PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION International Bureau



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6:		(11) International Publication Number: WO 97/04097							
C12N 15/12, 15/24, C07K 14/725, 14/54, A61K 38/17, 38/20, C12N 5/10, C07K 16/28	A2	(43) International Publication Date: 6 February 1997 (06.02.97							
(21) International Application Number: PCT/US9 (22) International Filing Date: 18 July 1996 (1		BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC,							
(30) Priority Data: 08/504,032 08/514,014 11 August 1995 (11.08.95)	-	Published S Without international search report and to be republished upon receipt of that report.							
(71) Applicant: GENETICS INSTITUTE, INC. [US/US]; bridge Park Drive, Cambridge, MA 02140 (US).	87 Car								
(72) Inventors: JACOBS, Kenneth; 151 Beaumont Avenuton, MA 02160 (US). KELLEHER, Kerry; 50 Hurle Marlborough, MA 01752 (US). CARLIN, McKet Chauncy Street #2, Cambridge, MA 02138 (US). MAN, Samuel; 9 Mohawk Drive, Acton, MA 017 PITTMAN, Debra; 20 N. Shore Road, Windham, N (US). MI, Sha; 4 Vernon Road, Belmont, MA 021 NEBEN, Steven; 13 Duggan Road, Acton, MA 017 GIANNOTTI, JoAnn; 409 Arlington Street, Act 01720 (US). GOLDEN-FLEET, Margaret; 19 Ush Medford, MA 02155 (US).	ey Circleough; GOLI 20 (US 74 0301 78 (US 20 (US ton, M	6. 6. 9. 1. 7. 1. 1. 1. 1.							
(74) Agent: BROWN, Scott, A.; Genetics Institute, CambridgePark Drive, Cambridge, MA 02140 (US		7							
(54) Title: HUMAN CTLA-8 AND USES OF CTLA-8-R.	ELAT	ED PROTEINS							

(57) Abstract

Polynucleotides encoding human CTLA-8 and related proteins are disclosed. Human CTLA-8 proteins and methods for their production are also disclosed. Methods of treatment using human CTLA-8 proteins, rat CTLA-8 proteins and herpesvirus herpes CTLA-8 proteins are also provided.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AM	Armenia	GB	United Kingdom	MW	Malawi
ΑT	Austria	GE	Georgia	MX	Mexico
AU	Australia	GN	Guinea	NE	Niger
BB	Barbados	GR	Greece	NL	Netherlands
BE	Belgium	HU	Hungary	NO	Norway
BF	Burkina Faso	IE	Ireland	NZ	New Zealand
BG	Bulgaria	IT	Italy	PL	Poland
BJ	Benin	JP	Japan	PT	Portugal
BR	Brazil	KE	Kenya	RO	Romania
BY	Belarus	KG	Kyrgysian	RU	Russian Federation
CA	Canada	KP	Democratic People's Republic	SD	Sudan
CF	Central African Republic		of Korea	SE	Sweden
CG	Congo	KR	Republic of Korea	SG	Singapore
CH	Switzerland	KZ	Kazakhstan	SI	Slovenia
a	Côte d'Ivoire	u	Liechtenstein	SK	Slovakia
CM	Cameroon	LK	Sri Lanka	SN	Senegal
CN	China	LR	Liberia	SZ	Swaziland
CS	Czechoslovakia	LT	Lithuania	770	Chad
CZ	Czech Republic	LU	Luxembourg	TG	Togo
DE	Germany	LV	Letvia	TJ	Tajikistan
DK	Denmark	MC	Monaco	Ϋ́T	Trinidad and Tobago
EE	Estonia	MD	Republic of Moldova	UA	Ukraine
ES	Spain	MG	Madagascar	UG	Uganda
FI	Pinland	ML	Mali	US	United States of America
FR	France	MN	Mongolia	UZ	Uzbekistan
GA	Gabon	MR	Mauritania	VN	Viet Nam

HUMAN CTLA-8 AND USES OF CTLA-8-RELATED PROTEINS

This application is a continuation-in-part of application Ser. No. 08/504,032, filed July 19, 1995, and a continuation-in-part of application Ser. No. 08/514,014, filed August 11, 1995.

Field of the Invention

The present

5

10

15

20

5

The present invention felates to human CTLA-8 proteins, nucleic acids encoding such proteins, methods of treatment using such proteins. The invention also relates to the use of rat CTLA-8 proteins and herpesvirus Saimiri ORF13 proteins in methods of treatment.

Background of the Invention

Cytokines are secreted proteins which act on specific hematopoietic target cells to cause a differentiation event or on other target cells to induce a particular physiological response, such as secretion of proteins characteristic of inflammation. Cytokines, also variously known as lymphokines, hematopoietins, interleukins, colony stimulating factors, and the like, can be important therapeutic agents, especially for diseases or conditions in which a specific cell population is depleted. For example, erythropoietin, G-CSF, and GM-CSF, have all become important for treatment of anemia and leukopenia, respectively. Other cytokines such as interleukin-3, interleukin-6, interleukin-11 and interleukin-12 show promise in treatment of conditions such as thrombocytopenia and modulation of immune response.

For these reasons a significant research effort has been expended in searching for novel cytokines and cloning the DNAs which encode them. In the past, novel cytokines were identified by assaying a particular cell such as a bone marrow cell, for a measurable response, such as proliferation. The search for novel cytokines has thus been limited by the assays available, and if a novel cytokine has an activity which is unmeasurable by a known assay, the cytokine remains undetectable. In a newer approach, cDNAs encoding cytokines have been detected using the polymerase chain

reaction (PCR) and oligonucleotide primers having homology to shared motifs of known cytokines or their receptors. The PCR approach is also limited by the necessity for knowledge of previously cloned cytokines in the same protein family. Cytokines have also been cloned using subtractive hybridization to construct and screen cDNA libraries, or they can potentially be cloned using PCR followed by gel electrophoresis to detect differentially expressed genes. The subtractive hybridization methods are based on the assumption that cytokine mRNAs are those that are differentially expressed, and these methods do not require any prior knowledge of the sequence of interest. However, many cytokines may be encoded by mRNAs which are not differentially expressed, and thus are undetectable using these methods.

It would be desirable to develop new methods for identifying novel cytokines and other secreted factors and to isolate polynucleotides encoding them.

Summary of the Invention

5

10

15

20

25

30

In developing the present invention, methods were employed which selectively identify polynucleotides which encode secreted proteins. One such polynucleotide was isolated which encodes "human CTLA-8." In accordance with the present invention, polynucleotides encoding human CTLA-8 and active fragments thereof are disclosed. "CTLA-8" is used throughout the present specification to refer to both

polynucleotides from all mammalian species.

In certain embodiments, the present invention provides an isolated polynucleotide comprising a nucleotide sequence selected from the group consisting of:

proteins and polynucleotides encoding those proteins and to refer to proteins and

- (a) the nucleotide sequence of SEQ ID NO:1 from nucleotide 146 to nucleotide 544;
- (b) a nucleotide sequence capable of hybridizing to a nucleic acid sequence specified in (a);
- (c) a nucleotide sequence varying from the sequence of the nucleotide sequence specified in (a) as a result of degeneracy of the genetic code; and
 - (d) an allelic variant of the nucleotide sequence specified in (a).

Preferably, the polynucleotide of the invention encodes a protein having CTLA-8 activity. In other embodiments the polynucleotide is operably linked to an expression control sequence. In other preferred embodiments, the polynucleotide is contained in a vector suitable for *in vivo* expression in a mammalian subject. Polynucleotides comprising the nucleotide sequence of SEQ ID NO:1 from nucleotide 55 to nucleotide 544, the nucleotide sequence of SEQ ID NO:1 from nucleotide 139 to nucleotide 544 or the nucleotide sequence of SEQ ID NO:1 from nucleotide 86 to nucleotide 544 are particularly preferred.

5

10

15

20

Host cells transformed with the polynucleotides of the invention are also provided, including mammalian cells.

Processes are also provided for producing a human CTLA-8 protein, said processes comprising:

- (a) growing a culture of the host cell of the invention in a suitable culture medium; and
- (b) purifying the human CTLA-8 protein from the culture.

Isolated human CTLA-8 protein is also provided which comprising an amino acid sequence selected from the group consisting of:

- (a) the amino acid sequence of SEQ ID NO:2;
- (b) the amino acid sequence of SEQ ID NO:2 from amino acids 11 to 163;
- (c) the amino acid sequence of SEQ ID NO:2 from amino acids 29 to 163;
- (d) the amino acid sequence of SEQ ID NO:2 from amino acids 31 to 163;and
 - (e) fragments of (a), (b), (c) or (d) having CTLA-8 activity.
- Proteins comprising the amino acid sequence of SEQ ID NO:2 and comprising the sequence from amino acids 29 to 163, from amino acid 31 to 163, or from amino acids 11 to 163 of SEQ ID NO:2 are particularly preferred. Preferably, the protein has CTLA-8 activity. Pharmaceuticals composition comprising a human CTLA-8 protein of the invention and a pharmaceutically acceptable carrier are also provided.
- 30 Compositions are also disclosed which comprise an antibody which specifically reacts with a human CTLA-8 protein of the invention.

Methods of treating a mammalian subject are also provided which comprise administering a therapeutically effective amount of a pharmaceutical composition comprising a human CTLA-8 protein.

Rat CTLA-8 and active (i.e., having CTLA-8 activity) fragments thereof may also be used in such methods of treatment. Preferably the rat protein is administered as a composition comprising a pharmaceutically acceptable carrier and a protein comprising an amino acid sequence selected from the group consisting of:

(a) the amino acid sequence of SEQ ID NO:4;

5

10

15

20

25

30

- (b) the amino acid sequence of SEQ ID NO:4 from amino acids 18 to 150; and
 - (c) fragments of (a) or (b) having CTLA-8 activity.

Herpesvirus Saimiri ORF13, referred to herein as "herpes CTLA-8", and active (i.e., having CTLA-8 activity) fragments thereof and active fragments thereof may also be used in such methods of treatment. Preferably the herpes CTLA-8 protein is administered as a composition comprising a pharmaceutically acceptable carrier and a protein comprising an amino acid sequence selected from the group consisting of:

- (a) the amino acid sequence of SEQ ID NO:6;
- (b) the amino acid sequence of SEQ ID NO:6 from amino acids 19 to 151;and
- (c) fragments of (a) or (b) having CTLA-8 activity.

The invention also provides a method of treating a mammalian subject comprising administering a therapeutically effective amount of a composition comprising a pharmaceutically acceptable carrier and IL-17 or an active fragment thereof.

In methods of treatment provided by the present invention, preferably the subject is treated to produce an effect selected from the group consisting of inhibition of angiogenesis, inhibition of growth or proliferation of vascular endothelial cells, inhibition of tumor growth, inhibition of angiogenesis-dependent tissue growth, proliferation of myeloid cells or progenitors, proliferation of erythroid cells or progenitors, proliferation of IFNγ production, induction of IL-3 production and induction of GM-CSF production.

Brief Description of the Figures

5

10

15

20

25

30

Fig. 1 is a comparison of homologous regions of the amino acid sequences of human CTLA-8 (indicated as "B18_F1"), rat CTLA-8 (indicated as "Musctla8") and herpes CTLA-8 (indicated as "Hsvie_2").

Fig. 2 depicts autoradiographs demonstrating expression of human CTLA-8 in COS cells.

Fig. 3 presents data relating to the ability of human CTLA-8 to inhibit angiogenesis.

Figs. 4 and 5 present data relating to the ability of human CTLA-8 to produce or induce hematopoietic activity.

Figs. 6 and 7 present data demonstrating the ability of human CTLA-8 to induce production of IL-6 and IL-8.

Detailed Description of Preferred Embodiments

The inventors of the present application have identified and provided a polynucleotide encoding a human CTLA-8 protein. SEQ ID NO:1 provides the nucleotide sequence of a cDNA encoding the human CTLA-8 protein. SEQ ID NO:2 provides the amino acid sequence of the human CTLA-8 protein. Alternatively, the initiating methionine may be at amino acid 11 of SEQ ID NO:2. On the basis of amino terminal sequencing, the mature protein sequence is believed to begin at amino acid 31 of SEQ ID NO:2 (encoded by the sequence beginning with nucleotide 146 of SEQ ID NO:1).

The region from amino acid 29 to amino acid 163 of human CTLA-8 (SEQ ID NO:2) shows marked homology to portions of rat CTLA-8 (amino acids 18 to 150 of SEQ ID NO:4) and herpesvirus Saimiri ORF13 ("herpes CTLA-8") (amino acids 19 to 151 of SEQ ID NO:5). A cDNA sequence encoding rat CTLA-8 is listed at SEQ ID NO:3 and its corresponding amino acid sequence is reported at SEQ ID NO:4. A cDNA sequence encoding herpes CTLA-8 is listed at SEQ ID NO:5 and its corresponding amino acid sequence is reported at SEQ ID NO:5 and its corresponding amino acid sequence is reported at SEQ ID NO:6. Homology between rat CTLA-8 and herpes CTLA-8 was reported by Rouvier et al., J. Immunol. 1993, 150, 5445-5456.

Applicants had previously incorrectly identified the rat sequences of SEQ ID NO:3 and SEQ ID NO:4 as applying to murine CTLA-8. Applicants' human CTLA-8 (B18) does also show homolgy to the true murine CTLA-8 sequence.

Golstein et al. (WO95/18826; Fossiez et al., Microbial Evasion and Subversion of Immunity 544:3222 (Abstract)) have also reported a species they initially identified as "human CTLA-8." However, examination of the sequence of the Golstein et al. species and the human CTLA-8 (B18) sequence of the present invention readily reveals that they are two different proteins, although they are homologous with each other and with the rat CTLA-8 and herpes CTLA-8 identified herein. The Golstein et al. species has now been renamed as interleukin-17 (IL-17). Because of the homology between applicants' human CTLA-8 (B18) and IL-17, these proteins are expected to share some activities.

5

10

15

20

25

30

It has also been preliminarily determined that human CTLA-8 (B18) forms homodimers when expressed. As a result, human CTLA-8 proteins may possess activity in either monomeric or dimeric forms. Human CTLA-8 proteins can also be produced as heterodimers with rat and herpes CTLA-8 proteins and with human IL-17. These heterodimers are also expected to have activities of the proteins of which they are comprised.

Forms of human CTLA-8 protein of less than full length are encompassed within the present invention and may be produced by expressing a corresponding fragment of the polynucleotide encoding the human CTLA-8 protein (SEQ ID NO:1). These corresponding polynucleotide fragments are also part of the present invention. Modified polynucleotides as described above may be made by standard molecular biology techniques, including site-directed mutagenesis methods which are known in the art or by the polymerase chain reaction using appropriate oligonucleotide primers.

For the purposes of the present invention, a protein has "CTLA-8 activity" if it either (1) displays biological activity in a factor-dependent cell proliferation assay (preferably an assay in which full-length the corresponding species full-length CTLA-8 is active) (including without limitation those assays described below), or (2) induces expression or secretion of γ -IFN, or (3) displays chemoattractant of chemotactic activity in a chemoattraction or chemotaxis assay (preferably as assay in which full-

length the corresponding species full-length CTLA-8 is active) or (4) induces expression of secretion of IL-3 or GM-CSF.

Human CTLA-8 protein or fragments thereof having CTLA-8 activity may be fused to carrier molecules such as immunoglobulins. For example, human CTLA-8 protein may be fused through "linker" sequences to the Fc portion of an immunoglobulin.

5

10

15

20

25

30

The invention also encompasses allelic variations of the nucleotide sequence as set forth in SEQ ID NO:1, that is, naturally-occurring alternative forms of the isolated polynucleotide of SEQ ID NO:1 which also encode human CTLA-8 or CTLA-8 proteins having CTLA-8 activity. Also included in the invention are isolated polynucleotides which hybridize to the nucleotide sequence set forth in SEQ ID NO:1 under highly stringent (0.2xSSC at 65°C), stringent (e.g. 4xSSC at 65°C or 50% formamide and 4xSSC at 42°C), or relaxed (4xSSC at 50°C or 30-40% formamideand 4xSSC at 42°C) conditions. Isolated polynucleotides which encode human CTLA-8 protein but which differ from the nucleotide sequence set forth in SEQ ID NO:1 by virtue of the degeneracy of the genetic code are also encompassed by the present invention. Variations in the nucleotide sequence as set forth in SEQ ID NO:1 which are caused by point mutations or by induced modifications which enhance CTLA-8 activity, half-life or production level are also included in the invention.

The isolated polynucleotides of the invention may be operably linked to an expression control sequence such as the pMT2 or pED expression vectors disclosed in Kaufman et al., Nucleic Acids Res. 19, 4485-4490 (1991), in order to produce the CTLA-8 protein recombinantly. Many suitable expression control sequences are known in the art. General methods of expressing recombinant proteins are also known and are exemplified in R. Kaufman, Methods in Enzymology 185, 537-566 (1990). As defined herein "operably linked" means enzymatically or chemically ligated to form a covalent bond between the isolated polynucleotide of the invention and the expression control sequence, in such a way that the CTLA-8 protein is expressed by a host cell which has been transformed (transfected) with the ligated polynucleotide/expression control sequence.

A number of types of cells may act as suitable host cells for expression of the human CTLA-8 protein. Any cell type capable of expressing functional human CTLA-8 protein may be used. Suitable mammalian host cells include, for example, monkey COS cells, Chinese Hamster Ovary (CHO) cells, human kidney 293 cells, human epidermal A431 cells, human Colo205 cells, 3T3 cells, CV-1 cells, other transformed primate cell lines, normal diploid cells, cell strains derived from in vitro culture of primary tissue, primary explants, HeLa cells, mouse L cells, BHK, HL-60, U937, HaK, Rat2, BaF3, 32D, FDCP-1, PC12 or C2C12 cells.

5

10

15

20

25

30

The human CTLA-8 protein may also be produced by operably linking the isolated polynucleotide of the invention to suitable control sequences in one or more insect expression vectors, and employing an insect expression system. Materials and methods for baculovirus/insect cell expression systems are commercially available in kit form from, e.g., Invitrogen, San Diego, California, U.S.A. (the MaxBac® kit), and such methods are well known in the art, as described in Summers and Smith, Texas Agricultural Experiment Station Bulletin No. 1555 (1987), incorporated herein by reference. Soluble forms of the human CTLA-8 protein may also be produced in insect cells using appropriate isolated polynucleotides as described above.

Alternatively, the human CTLA-8 protein may be produced in lower eukaryotes such as yeast or in prokaryotes such as bacteria. Suitable yeast strains include Saccharomyces cerevisiae, Schizosaccharomyces pombe, Kluyveromyces strains, Candida, or any yeast strain capable of expressing heterologous proteins. Suitable bacterial strains include Escherichia coli, Bacillus subtilis, Salmonella typhimurium, or any bacterial strain capable of expressing heterologous proteins.

The human CTLA-8 protein of the invention may also be expressed as a product of transgenic animals, e.g., as a component of the milk of transgenic cows, goats, pigs, or sheep which are characterized by somatic or germ cells containing a polynucleotide sequence encoding the human CTLA-8 protein.

The human CTLA-8 protein of the invention may be prepared by growing a culture of transformed host cells under culture conditions necessary to express the desired protein. The resulting expressed protein may then be purified from the culture medium or cell extracts. Soluble forms of the human CTLA-8 protein of the invention

can be purified from conditioned media. Membrane-bound forms of human CTLA-8 protein of the invention can be purified by preparing a total membrane fraction from the expressing cell and extracting the membranes with a non-ionic detergent such as Triton X-100.

5

10

15

20

25

30

The human CTLA-8 protein can be purified using methods known to those skilled in the art. For example, the human CTLA-8 protein of the invention can be concentrated using a commercially available protein concentration filter, for example, an Amicon or Millipore Pellicon ultrafiltration unit. Following the concentration step, the concentrate can be applied to a purification matrix such as a gel filtration medium. Alternatively, an anion exchange resin can be employed, for example, a matrix or substrate having pendant diethylaminoethyl (DEAE) groups. The matrices can be acrylamide, agarose, dextran, cellulose or other types commonly employed in protein purification. Alternatively, a cation exchange step can be employed. Suitable cation exchangers include various insoluble matrices comprising sulfopropyl or carboxymethyl groups. Sulfopropyl groups are preferred (e.g., S-Sepharose® columns). The purification of the human CTLA-8 protein from culture supernatant may also include one or more column steps over such affinity resins as concanavalin A-agarose, heparin-toyopearl® or Cibacrom blue 3GA Sepharose®; or by hydrophobic interaction chromatography using such resins as phenyl ether, butyl ether, or propyl ether; or by immunoaffinity chromatography. Finally, one or more reversephase high performance liquid chromatography (RP-HPLC) steps employing hydrophobic RP-HPLC media, e.g., silica gel having pendant methyl or other aliphatic groups, can be employed to further purify the human CTLA-8 protein. Some or all of the foregoing purification steps, in various combinations or with other known methods, can also be employed to provide a substantially purified isolated recombinant protein.

Preferably, the human CTLA-8 protein is purified so that it is substantially free of other mammalian proteins.

It is believed that human CTLA-8, active fragments and variants thereof, and CTLA-8 related proteins (such as, for example, rat CTLA-8 and herpes CTLA-8) (collectively "CTLA-8 proteins") possess or induce cytokine activities. Human

CTLA-8 expression correlated with γ-IFN expression in induced primary cells and can induce the expression of IL-3 and/or GM-CSF, which expression can in turn produce effects associated with the induced cytokine. Therefore, human CTLA-8 and CTLA-8 related proteins may have an effect on proliferation or function of myeloid cells, erythroid cells, lymphoid cells and their progenitors. Human CTLA-8 proteins may also play a role in formation of platelets or their progenitors.

5

10

15

20

25

30

A protein of the present invention may exhibit cytokine, cell proliferation (either inducing or inhibiting) or cell differentiation (either inducing or inhibiting) activity or may induce production of other cytokines in certain cell populations. Many protein factors discovered to date, including all known cytokines, have exhibited activity in one or more factor dependent cell proliferation assays, and hence the assays serve as a convenient confirmation of cytokine activity. The activity of a protein of the present invention is evidenced by any one of a number of routine factor dependent cell proliferation assays for cell lines including, without limitation, 32D, DA2, DA1G, T10, B9, B9/11, BaF3, MC9/G, M+ (preB M+), 2E8, RB5, DA1, 123, T1165, HT2, CTLL2, TF-1, Mo7e and CMK.

The activity of a protein of the invention may, among other means, be measured by the following methods:

Assays for T-cell or thymocyte proliferation include without limitation those described in: Current Protocols in Immunology, Ed by J. E. Coligan, A.M. Kruisbeek, D.H. Margulies, E.M. Shevach, W Strober, Pub. Greene Publishing Associates and Wiley-Interscience (Chapter 3, In Vitro assays for Mouse Lymphocyte Function 3.1-3.19; Chapter 7, Immunologic studies in Humans); Takai et al., J. Immunol. 137:3494-3500, 1986; Bertagnolli et al., J. Immunol. 145:1706-1712, 1990; Bertagnolli et al., Cellular Immunology 133:327-341, 1991; Bertagnolli, et al., J. Immunol. 149:3778-3783, 1992; Bowman et al., J. Immunol. 152: 1756-1761, 1994.

Assays for cytokine production and/or proliferation of spleen cells, lymph node cells or thymocytes include, without limitation, those described in: Polyclonal T cell stimulation, Kruisbeek, A.M. and Shevach, E.M. In *Current Protocols in Immunology*. J.E.e.a. Coligan eds. Vol 1 pp. 3.12.1-3.12.14, John Wiley and Sons, Toronto. 1994; and Measurement of mouse and human Interferon γ, Schreiber, R.D.

In Current Protocols in Immunology. J.E.e.a. Coligan eds. Vol 1 pp. 6.8.1-6.8.8, John Wiley and Sons, Toronto. 1994.

5

10

15

20

25

30

Assays for proliferation and differentiation of hematopoietic and lymphopoietic cells include, without limitation, those described in: Measurement of Human and Murine Interleukin 2 and Interleukin 4, Bottomly, K., Davis, L.S. and Lipsky, P.E. In Current Protocols in Immunology. J.E.e.a. Coligan eds. Vol 1 pp. 6.3.1-6.3.12, John Wiley and Sons, Toronto. 1991; deVries et al., J. Exp. Med. 173:1205-1211, 1991; Moreau et al., Nature 336:690-692, 1988; Greenberger et al., Proc. Natl. Acad. Sci. U.S.A. 80:2931-2938, 1983; Measurement of mouse and human interleukin 6 - Nordan, R. In Current Protocols in Immunology. J.E.e.a. Coligan eds. Vol 1 pp. 6.6.1-6.6.5, John Wiley and Sons, Toronto. 1991; Smith et al., Proc. Natl. Acad. Sci. U.S.A. 83:1857-1861, 1986; Measurement of human Interleukin 11 - Bennett, F., Giannotti, J., Clark, S.C. and Turner, K. J. In Current Protocols in Immunology. J.E.e.a. Coligan eds. Vol 1 pp. 6.15.1 John Wiley and Sons, Toronto. 1991; Measurement of mouse and human Interleukin 9 - Ciarletta, A., Giannotti, J., Clark, S.C. and Turner, K.J. In Current Protocols in Immunology. J.E.e.a. Coligan eds. Vol 1 pp. 6.13.1, John Wiley and Sons, Toronto. 1991.

Assays for T-cell clone responses to antigens (which will identify, among others, proteins that affect APC-T cell interactions as well as direct T-cell effects by measuring proliferation and cytokine production) include, without limitation, those described in: Current Protocols in Immunology, Ed by J. E. Coligan, A.M. Kruisbeek, D.H. Margulies, E.M. Shevach, W Strober

Pub. Greene Publishing Associates and Wiley-Interscience (Chapter 3, In Vitro assays for Mouse Lymphocyte Function; Chapter 6, Cytokines and their cellular receptors; Chapter 7, Immunologic studies in Humans); Weinberger et al., Proc. Natl. Acad. Sci. USA 77:6091-6095, 1980; Weinberger et al., Eur. J. Immun. 11:405-411, 1981; Takai et al., J. Immunol. 137:3494-3500, 1986; Takai et al., J. Immunol. 140:508-512, 1988.

A protein of the present invention may also exhibit immune stimulating or immune suppressing activity, including without limitation the activities for which assays are described herein. A protein may be useful in the treatment of various

immune deficiencies and disorders (including severe combined immunodeficiency (SCID)), e.g., in regulating (up or down) growth and proliferation of T and/or B lymphocytes, as well as effecting the cytolytic activity of NK cells and other cell populations. These immune deficiencies may be genetic or be caused by viral (e.g., HIV) as well as bacterial or fungal infections, or may result from autoimmune disorders. More specifically, infectious diseases causes by viral, bacterial, fungal or other infection may be treatable using a protein of the present invention, including infections by HIV, hepatitis viruses, herpes viruses, mycobacteria, leshmania, malaria and various fungal infections such as candida. Of course, in this regard, a protein of the present invention may also be useful where a boost to the immune system generally would be indicated, i.e., in the treatment of cancer.

5

ĺŨ

15

20

25

30

Autoimmune disorders which may be treated using a protein of the present invention include, for example, multiple sclerosis, systemic lupus erythematosus, rheumatoid arthritis, autoimmune pulmonary inflammation, Guillain-Barre syndrome, autoimmune thyroiditis, insulin dependent diabetes mellitis, myasthenia gravis, graft-versus-host disease and autoimmune inflammatory eye disease. Such a protein of the present invention may also to be useful in the treatment of allergic reactions and conditions, such as asthma or other respiratory problems. Other conditions, in which immune suppression is desired (including, for example, asthma and related respiratory conditions), may also be treatable using a protein of the present invention.

A protein of the present invention may also suppress chronic or acute inflammation, such as, for example, that associated with infection (such as septic shock or systemic inflammatory response syndrome (SIRS)), inflammatory bowel disease, Crohn's disease or resulting from over production of cytokines such as TNF or IL-1 (such as the effect demonstrated by IL-11).

The activity of a protein of the invention may, among other means, be measured by the following methods:

Suitable assays for thymocyte or splenocyte cytotoxicity include, without limitation, those described in: Current Protocols in Immunology, Ed by J. E. Coligan, A.M. Kruisbeek, D.H. Margulies, E.M. Shevach, W Strober, Pub. Greene Publishing Associates and Wiley-Interscience (Chapter 3, In Vitro assays for Mouse Lymphocyte

Function 3.1-3.19; Chapter 7, Immunologic studies in Humans); Herrmann et al., Proc. Natl. Acad. Sci. USA 78:2488-2492, 1981; Herrmann et al., J. Immunol. 128:1968-1974, 1982; Handa et al., J. Immunol. 135:1564-1572, 1985; Takai et al., J. Immunol. 137:3494-3500, 1986; Takai et al., J. Immunol. 140:508-512, 1988; Herrmann et al., Proc. Natl. Acad. Sci. USA 78:2488-2492, 1981; Herrmann et al., J. Immunol. 128:1968-1974, 1982; Handa et al., J. Immunol. 135:1564-1572, 1985; Takai et al., J. Immunol. 137:3494-3500, 1986; Bowmanet al., J. Virology 61:1992-1998; Takai et al., J. Immunol. 140:508-512, 1988; Bertagnolli et al., Cellular Immunology 133:327-341, 1991; Brown et al., J. Immunol. 153:3079-3092, 1994.

5

10

15

20

25

30

Assays for T-cell-dependent immunoglobulin responses and isotype switching (which will identify, among others, proteins that modulate T-cell dependent antibody responses and that affect Th1/Th2 profiles) include, without limitation, those described in: Maliszewski, J. Immunol. 144:3028-3033, 1990; and Assays for B cell function: *In vitro* antibody production, Mond, J.J. and Brunswick, M. In *Current Protocols in Immunology*. J.E.e.a. Coligan eds. Vol 1 pp. 3.8.1-3.8.16, John Wiley and Sons, Toronto. 1994.

Mixed lymphocyte reaction (MLR) assays (which will identify, among others, proteins that generate predominantly Th1 and CTL responses) include, without limitation, those described in: Current Protocols in Immunology, Ed by J. E. Coligan, A.M. Kruisbeek, D.H. Margulies, E.M. Shevach, W Strober, Pub. Greene Publishing Associates and Wiley-Interscience (Chapter 3, In Vitro assays for Mouse Lymphocyte Function 3.1-3.19; Chapter 7, Immunologic studies in Humans); Takai et al., J. Immunol. 137:3494-3500, 1986; Takai et al., J. Immunol. 140:508-512, 1988; Bertagnolli et al., J. Immunol. 149:3778-3783, 1992.

Dendritic cell-dependent assays (which will identify, among others, proteins expressed by denritic cells that activate naive T-cells) include, without limitation, those described in: Guery et al., J. Immunol. 134:536-544, 1995; Inaba et al., Journal of Experimenal Medicine 173:549-559, 1991; Macatonia et al., Journal of Immunology 154:5071-5079, 1995; Porgador et al., Journal of Experimental Medicine 182:255-260, 1995; Nair et al., Journal of Virology 67:4062-4069, 1993; Huang et al., Science 264:961-965, 1994; Macatonia et al., Journal of Experimental Medicine

169:1255-1264, 1989; Bhardwaj et al., Journal of Clinical Investigation 94:797-807, 1994; and Inaba et al., Journal of Experimental Medicine 172:631-640, 1990.

Assays for lymphocyte survival/apoptosis (which will identify, among others, proteins that prevent apoptosis after superantigen induction and proteins that regulate lymphocyte homeostasis) include, without limitation, those described in: Darzynkiewicz et al., Cytometry 13:795-808, 1992; Gorczyca et al., Leukemia 7:659-670, 1993; Gorczyca et al., Cancer Research 53:1945-1951, 1993; Itoh et al., Cell 66:233-243, 1991; Zacharchuk, Journal of Immunology 145:4037-4045, 1990; Zamai et al., Cytometry 14:891-897, 1993; Gorczyca et al., International Journal of Oncology 1:639-648, 1992.

5

10

15

20

25

30

Assays for proteins that influence early steps of T-cell commitment and development include, without limitation, those described in: Antica et al., Blood 84:111-117, 1994; Fine et al., Cellular Immunology 155:111-122, 1994; Galy et al., Blood 85:2770-2778, 1995; Toki et al., Proc. Nat. Acad Sci. USA 88:7548-7551, 1991.

A protein of the present invention may be useful in regulation of hematopoiesis and, consequently, in the treatment of myeloid or lymphoid cell deficiencies. Even marginal biological activity in support of colony forming cells or of factor-dependent cell lines indicates involvement in regulating hematopoiesis, e.g. in supporting the growth and proliferation of erythroid progenitor cells alone or in combination with other cytokines, thereby indicating utility, for example, in treating various anemias or for use in conjunction with irradiation/chemotherapy to stimulate the production of erythroid precursors and/or erythroid cells; in supporting the growth and proliferation of myeloid cells such as granulocytes and monocytes/macrophages (i.e., traditional CSF activity) useful, for example, in conjunction with chemotherapy to prevent or treat consequent myelo-suppression; in supporting the growth and proliferation of megakaryocytes and consequently of platelets thereby allowing prevention or treatment of various platelet disorders such as thrombocytopenia, and generally for use in place of or complimentarily to platelet transfusions; and/or in supporting the growth and proliferation of hematopoietic stem cells which are capable of maturing to any and all of the above-mentioned hematopoietic cells and therefore find therapeutic utility

in various stem cell disorders (such as those usually treated with transplantation, including, without limitation, aplastic anemia and paroxysmal nocturnal hemoglobinuria), as well as in repopulating the stem cell compartment post irradiation/chemotherapy, either *in-vivo* or *ex-vivo* (i.e. in conjunction with bone marrow transplantation) as normal cells or genetically manipulated for gene therapy.

The activity of a protein of the invention may, among other means, be measured by the following methods:

Suitable assays for proliferation and differentiation of various hematopoietic lines are cited above.

10

5

Assays for embryonic stem cell differentiation (which will identify, among others, proteins that influence embyronic differentiation hematopoiesis) include, without limitation, those described in: Johansson et al. Cellular Biology 15:141-151, 1995; Keller et al., Molecular and Cellular Biology 13:473-486, 1993; McClanahan et al., Blood 81:2903-2915, 1993.

15

20

25

30

Assays for stem cell survival and differentiation (which will identify, among others, proteins that regulate lympho-hematopoiesis) include, without limitation, those described in: Methylcellulose colony forming assays, Freshney, M.G. In Culture of Hematopoietic Cells: R.I. Freshney, et al. eds. Vol pp. 265-268, Wiley-Liss, Inc., New York, NY. 1994; Hirayama et al., Proc. Natl. Acad. Sci. USA 89:5907-5911, 1992; Primitive hematopoietic colony forming cells with high proliferative potential, McNiece, I.K. and Briddell, R.A. In Culture of Hematopoietic Cells. R.I. Freshney, et al. eds. Vol pp. 23-39, Wiley-Liss, Inc., New York, NY. 1994; Neben et al., Experimental Hematology 22:353-359, 1994; Cobblestone area forming cell assay, Ploemacher, R.E. In Culture of Hematopoietic Cells. R.I. Freshney, et al. eds. Vol pp. 1-21, Wiley-Liss, Inc.., New York, NY. 1994; Long term bone marrow cultures in the presence of stromal cells, Spooncer, E., Dexter, M. and Allen, T. In Culture of Hematopoietic Cells. R.I. Freshney, et al. eds. Vol pp. 163-179, Wiley-Liss, Inc., New York, NY. 1994; Long term culture initating cell assay, Sutherland, H.J. In Culture of Hematopoietic Cells. R.I. Freshney, et al. eds. Vol pp. 139-162, Wiley-Liss, Inc., New York, NY. 1994.

CTLA-8 proteins are useful in the treatment of various immune deficiencies and disorders (including SCID), e.g., in regulating (up or down) growth, proliferation and/or activity of T and/or B lymphocytes, as well as the cytolytic activity of NK cells: These immune deficiencies may be caused by viral (e.g., HIV) as well as bacterial infections, or may result from autoimmune disorders. More specifically, infectious diseases caused by viral, bacterial, fungal or other infection may be treatable using CTLA-8 proteins, including infections by HIV, hepatitis, influenza, CMV, herpes, mycobacterium, leishmaniasis, malaria and various fungal infections (such as candida). Of course, in this regard, the CTLA-8 proteins may also be useful where a boost to the immune system generally would be indicated, i.e., in the treatment of cancer or as an adjuvant to vaccines. Autoimmune disorders which may be treated using factors of the present invention include, for example, multiple sclerosis, systemic lupus erythematosus, rheumatoid arthritis, autoimmune pulmonary inflammation, Guillain-Barre syndrome, autoimmune thyroiditis, insulin dependent diabetes melitis and autoimmune inflammatory eye disease. The CTLA-8 proteins are also expected to be useful in the treatment of allergic reactions and conditions.

5

1Û

15

20

25

30

CTLA-8 proteins are also expected to have chemotactic activity. A protein or peptide has "chemotactic activity," as used herein, if it can stimulate, directly or indirectly, the directed orientation or movement of cells, including myeloid and lymphoid cells. Preferably, the protein or peptide has the ability to directly stimulate directed movement of cells (particularly T-cells). Whether a particular protein or peptide has chemotactic activity for cells can be readily determined by employing such protein or peptide in any known assay for cell chemotaxis.

CTLA-8 proteins also inhibit growth and proliferation of vascular endothelial cells. As a result, human CTLA-8 proteins are effective in inhibiting angiogenesis (i.e., vascular formation). This activity will also be useful in the treatment of tumors and other conditions in which angiogenesis in involved. Inhibition of angiogenesis by human CTLA-8 proteins will also result in inhibition or prevention of the condition to which normal angiogenesis would contribute.

Isolated CTLA-8 proteins, purified from cells or recombinantly produced, may be used as a pharmaceutical composition when combined with a pharmaceutically

acceptable carrier. Such a composition may contain, in addition to CTLA-8 protein and carrier, diluents, fillers, salts, buffers, stabilizers, solubilizers, and other materials well known in the art. The term "pharmaceutically acceptable" means a non-toxic material that does not interfere with the effectiveness of the biological activity of the active ingredient(s). The characteristics of the carrier will depend on the route of administration. The pharmaceutical composition of the invention may also contain cytokines, lymphokines, or other hematopoietic factors such as M-CSF, GM-CSF, IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, IL-15, G-CSF, y-IFN, stem cell factor, and erythropoietin. The pharmaceutical composition may contain thrombolytic or anti-thrombotic factors such as plasminogen activator and Factor VIII. The pharmaceutical composition may further contain other antiinflammatory agents. Such additional factors and/or agents may be included in the pharmaceutical composition to produce a synergistic effect with CTLA-8 protein, or to minimize side effects caused by the CTLA-8 protein. Conversely, CTLA-8 protein may be included in formulations of the particular cytokine, lymphokine, other hematopoietic factor, thrombolytic or anti-thrombotic factor, or anti-inflammatory agent to minimize side effects of the cytokine, lymphokine, other hematopoietic factor, thrombolytic or anti-thrombotic factor, or anti-inflammatory agent.

5

10

15

20

25

30

The pharmaceutical composition of the invention may be in the form of a liposome in which CTLA-8 protein is combined, in addition to other pharmaceutically acceptable carriers, with amphipathic agents such as lipids which exist in aggregated form as micelles, insoluble monolayers, liquid crystals, or lamellar layers which in aqueous solution. Suitable lipids for liposomal formulation include, without limitation, monoglycerides, diglycerides, sulfatides, lysolecithin, phospholipids, saponin, bile acids, and the like. Preparation of such liposomal formulations is within the level of skill in the art, as disclosed, for example, in U.S. Patent No. 4,235,871; U.S. Patent No. 4,501,728; U.S. Patent No. 4,837,028; and U.S. Patent No. 4,737,323, all of which are incorporated herein by reference.

As used herein, the term "therapeutically effective amount" means the total amount of each active component of the pharmaceutical composition or method that is sufficient to show a meaningful patient benefit, e.g., amelioration of symptoms of,

healing of, or increase in rate of healing of such conditions. When applied to an individual active ingredient, administered alone, the term refers to that ingredient alone. When applied to a combination, the term refers to combined amounts of the active ingredients that result in the therapeutic effect, whether administered in combination, serially or simultaneously.

5

10

15

20

25

30

In practicing the method of treatment or use of the present invention, a therapeutically effective amount of CTLA-8 protein is administered to a mammal. CTLA-8 protein may be administered in accordance with the method of the invention either alone or in combination with other therapies such as treatments employing cytokines, lymphokines or other hematopoietic factors. When co-administered with one or more cytokines, lymphokines, other hematopoietic factors or vaccine components (such as antigens or other adjuvants), CTLA-8 protein may be administered either simultaneously with the cytokine(s), lymphokine(s), other hematopoietic factor(s), thrombolytic or anti-thrombotic factors, or sequentially. If administered sequentially, the attending physician will decide on the appropriate sequence of administering CTLA-8 protein in combination with cytokine(s), lymphokine(s), other hematopoietic factor(s), thrombolytic or anti-thrombotic factors.

Administration of CTLA-8 protein used in the pharmaceutical composition or to practice the method of the present invention can be carried out in a variety of conventional ways, such as oral ingestion, inhalation, or cutaneous, subcutaneous, or intravenous injection. Intravenous administration to the patient is preferred.

When a therapeutically effective amount of CTLA-8 protein is administered orally, CTLA-8 protein will be in the form of a tablet, capsule, powder, solution or elixir. When administered in tablet form, the pharmaceutical composition of the invention may additionally contain a solid carrier such as a gelatin or an adjuvant. The tablet, capsule, and powder contain from about 5 to 95% CTLA-8 protein, and preferably from about 25 to 90% CTLA-8 protein. When administered in liquid form, a liquid carrier such as water, petroleum, oils of animal or plant origin such as peanut oil, mineral oil, soybean oil, or sesame oil, or synthetic oils may be added. The liquid form of the pharmaceutical composition may further contain physiological saline solution, dextrose or other saccharide solution, or glycols such as ethylene glycol,

propylene glycol or polyethylene glycol. When administered in liquid form, the pharmaceutical composition contains from about 0.5 to 90% by weight of CTLA-8 protein, and preferably from about 1 to 50% CTLA-8 protein.

5

10

15

20

25

30

When a therapeutically effective amount of CTLA-8 protein is administered by intravenous, cutaneous or subcutaneous injection, CTLA-8 protein will be in the form of a pyrogen-free, parenterally acceptable aqueous solution. The preparation of such parenterally acceptable protein solutions, having due regard to pH, isotonicity, stability, and the like, is within the skill in the art. A preferred pharmaceutical composition for intravenous, cutaneous, or subcutaneous injection should contain, in addition to CTLA-8 protein an isotonic vehicle such as Sodium Chloride Injection, Ringer's Injection, Dextrose Injection, Dextrose and Sodium Chloride Injection, Lactated Ringer's Injection, or other vehicle as known in the art. The pharmaceutical composition of the present invention may also contain stabilizers, preservatives, buffers, antioxidants, or other additive known to those of skill in the art.

The amount of CTLA-8 protein in the pharmaceutical composition of the present invention will depend upon the nature and severity of the condition being treated, and on the nature of prior treatments which the patient has undergone. Ultimately, the attending physician will decide the amount of CTLA-8 protein with which to treat each individual patient. Initially, the attending physician will administer low doses of CTLA-8 protein and observe the patient's response. Larger doses of CTLA-8 protein may be administered until the optimal therapeutic effect is obtained for the patient, and at that point the dosage is not generally increased further. It is contemplated that the various pharmaceutical compositions used to practice the method of the present invention should contain about 0.1 µg to about 100 mg of CTLA-8 protein per kg body weight, preferably about 0.1 µg to about 100 µg of CTLA-8 protein per kg body weight, more preferably preferably about 0.1 µg to about 100 µg of CTLA-8 protein per kg body weight, most preferably preferably about 0.1 µg to about 100 µg of CTLA-8 protein per kg body weight.

The duration of intravenous therapy using the pharmaceutical composition of the present invention will vary, depending on the severity of the disease being treated and the condition and potential idiosyncratic response of each individual patient. It

is contemplated that the duration of each application of the CTLA-8 protein will be in the range of 12 to 24 hours of continuous intravenous administration. Ultimately the attending physician will decide on the appropriate duration of intravenous therapy using the pharmaceutical composition of the present invention.

5

10

15

20

25

30

CTLA-8 protein of the invention may also be used to immunize animals to obtain polyclonal and monoclonal antibodies which specifically react with the CTLA-8 protein and which may inhibit CTLA-8 binding to its receptor. Such antibodies are also useful for performing diagnostics assays for CTLA-8 in accordance with known methods. Such antibodies may be obtained using the entire CTLA-8 protein as an immunogen, or by using fragments of human CTLA-8 protein. The peptide immunogens additionally may contain a cysteine residue at the carboxyl terminus, and are conjugated to a hapten such as keyhole limpet hemocyanin (KLH). Additional peptide immunogens may be generated by replacing tyrosine residues with sulfated tyrosine residues. Methods for synthesizing such peptides are known in the art, for example, as in R.P. Merrifield, J.Amer.Chem.Soc. 85, 2149-2154 (1963); J.L. Krstenansky, et al., FEBS Lett. 211, 10 (1987).

Neutralizing or non-neutralizing antibodies (preferably monoclonal antibodies) binding to human CTLA-8 protein may also be useful therapeutics for certain tumors and also in the treatment of conditions described above. These neutralizing monoclonal antibodies are capable of blocking the ligand binding to the human CTLA-8 protein or mayh promote clearance of protein from the patient.

Because of their homology to human CTLA-8, rat CTLA-8 proteins, herpes CTLA-8 proteins and IL-17 proteins (the "human CTLA-8" of Golstein et al., supra) will also possess CTLA-8 activity as described above. As a result, rat and herpes CTLA-8 proteins and IL-17 proteins, as well as active fragments and variants thereof, can be used in preparation of pharmaceutical compositions and in methods of treatment as described for human CTLA-8. Rat and herpes CTLA-8 proteins, and active fragments and variants thereof, can be produced as described above using the polynucleotides (or fragments or variants thereof) described in SEQ ID NO:3 and SEQ ID NO:5, respectively. Rat and herpes CTLA-8 may also be produced as described in Rouvier et al., J. Immunol. 1993, 150, 5445-5456. CTLA-8 proteins of other

species can also be used as described herein. cDNAs encoding rat CTLA-8 and herpes CTLA-8 were deposited with the American Type Culture Collection on July 6, 1995 and assigned accession numbers ATCC 69867 and ATCC 69866, respectively. IL-17 proteins may also be produced as described in Golstein et al., supra.

Because of its homology to IL-17, the human CTLA-8 (B18) proteins of the present invention may also share some activities with IL-17.

5

10

15

20

25

30

For the purposes of treatment or therapy, any of the proteins discussed or disclosed herein may be administered by *in vivo* expression of the protein in a mammalian subject. In such instances, a polynucleotide encoding the desired protein is administered to the subject in manner allowing expression in accordance with known methods, including without limitation the adenovirus methods disclosed herein.

Example 1

Isolation of Human CTLA-8 cDNA

A partial clone for human CTLA-8 was isolated from a cDNA library made from RNA isolated from stimulated human peripheral blood mononuclear cells. This partial was identified as "B18." B18 is sometimes used herein to refer to the human CTLA-8 of the present invention. Homology searches identified this partial clone as being related to the herpes and rat CTLA-8 genes. DNA sequence of this partial clone was used to isolate the full-length clone.

In order to isolate a full-length cDNA for B18, a directional, full-length cDNA library by standard means in the COS expression vector pMV2. The cDNA library was transformed into *E. coli* by electroporation. The bulk of the original transformed cDNA library was frozen in glycerol at -80°C. An aliquot was titered to measure the concentration of transformed *E. coli*. The *E. coli* were thawed, diluted to 76,000/0.1 ml in media containing ampicillin, and 0.1 ml was distributed into the wells of a microtiter dish in an 8 x 8 array. The microtiter dish was placed at 37°C overnight to grow the *E. coli*.

To prepare DNA for PCR, 20 µl aliquots of culture from each well were withdrawn and pooled separately for each row and column of eight wells, giving 16

pools of 160 μ l each. The *E. coli* were pelleted, resuspended in 160 μ l of standard lysis buffer consisting of 10 mM TrisHCI pH8, 1 mM EDTA, 0.01% Triton X-100, and lysed by heating to 95°C for 10 minutes.

To identify which of the wells contained *E. coli* transformed with B18, PCR was performed first on the DNA preps corresponding to the eight columns. The PCR consisted of two sequential reactions with nested oligonucleotides using standard conditions. The oligonucleotides used for the PCR reaction were derived from the sequence of the partial B18 clone. They were:

5

10

15

20

25

30

B185: CACAGGCATACACAGGAAGATACATTCA (SEQ ID NO:7)

B183: TCTTGCTGGATGGGAACGGAATTCA (SEQ ID NO:8)

B18N: ATACATTCACAGAAGAGCTTCCTGCACA (SEQ ID NO:9)

The PCR conditions were 2.5 mM MgCl₂ and 95°C x 2 min for one cycle, 95°C x 1 min plus 68°C x 1 min for 30 cycles, and 68°C x 10 for one cycle. Each reaction was 20 μ l. The first reaction contained oligonucleotides B185 and B183 and 1 μ l of the DNA preparations. The second reaction contained oligonucleotides B183 and B18N and 1 μ l of the first reaction.

DNA preps that potentially contained a full-length B18 cDNA clone were identified by agarose gel electrophoresis on an aliquot of the second PCR reaction. A DNA band of the correct mobility was assumed to be derived from a B18 cDNA. Next the same sequence of PCR reactions and gel analysis was done on the DNA preps corresponding to the eight rows. The intersection of a row and a column identified well A2 as potentially containing B18, narrowing it down to the 76,000 E. coli originally seeded into that well.

To further purify the individual *E. coli* containing the putative full-length B18 cDNA clone, the concentration of *E. coli* in well A2 was measured by titering and plating dilutions of the well. Then 7600 *E. coli* were seeded into the wells of a second microtiter plate in an 8 x 8 array. The *E. coli* were grown overnight; wells were pooled, and DNA was prepared as described above. To identify which of these wells contained *E. coli* transformed with B18, sequential PCR reactions were performed

essentially as described above. Agarose gel electrophoresis identified well B2 as potentially containing a B18 cDNA.

The E. coli containing this cDNA was further purified by seeding wells of a microtiter plate with 253 E. coli per well and proceeding as for the purification of the E. coli in well A2. Well C3 was identified as containing a putative full-length B18 cDNA clone. The exact E. coli was identified by plating the contents of the well onto bacterial culture media and then screening the E. coli colonies following established protocols. The probe for these hybridizations was a PCR fragment generated by doing a PCR reaction on the B18 clone using as primers the oligonucleotides described above (SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9). When a single colony was identified, DNA was prepared and sequenced by standard methods. Comparison of this sequence to the sequence of the original partial clone confirmed identity and that the isolated cDNA was full-length.

The full-length clone was deposited with the American Type Culture Collection on July 6, 1995 and assigned accession number ATCC 69868.

Example 2

Expression of Human CTLA-8

The full-length B18 clone for human CTLA-8 was transfected into COS cells which were then labelled with ³⁵S-methionine. An aliquot of conditioned medium from the transfected cell culture was reduced, denatured and electrophoresed on polyacrylamide gels. Autoradiographs of those gels are reproduced in Fig. 2. The band indicated by the arrow demonstrates expression of human CTLA-8.

25 Example 3

5

10

15

20

30

Inhibition of Angiogenesis by Human CTLA-8

The ability of human CTLA-8 to inhibit angiogenesis was examined in an angiostatic activity assay (endothelial cell proliferation assay). The assay was done in a 96 well plate. Primary human umbilical cells (HUVECs) were seeded to $2x10^3$ cells per well in EGM medium (Clonetics)/20% FCS and incubated at 37° C for 24 hr. The cells were then starved in M199 medium (GIBCO BRL) containing 10% charcoal

treated serum (M199-CS) for 48 hr at 37°C. Conditioned media containing B18 (human CTLA-8) was obtained from transfected COS or stably expressing CHO cells and 1:10, 1:50, 1:250, and 1:1250 dilutions prepared in M199-CS medium containing 100 ng/ml FGF. The dilutions of B18 were added to the starved cells and incubated for 72 hr at 37°C. The cells were then radiolabeled by [³H]-thymidine for 6 hr. Radiolabeled cells were washed with PBS and trypsinized for liquid scintilation counting. Results were plotted using Kaleidograph software. The results are shown in Fig. 3. In the figure, "Med" is the mock control, "B18" and "B18-1" were conditioned medium from two independent transfections of COS with DNA encoding human CTLA-8 (B18). IFNγ was used as a positive control angiostatic (i.e., angiogenesis inhibition) activity. These data demonstrate that human CTLA-8 (B18) inhibits angiogenesis.

Example 4

15

10

5

Hematopoietic Activity of Human CTLA-8

The hematopoietic activity of human CTLA-8 (B18) expressed *in vivo* was examined by construction of a recombinant adenovirus vector.

The B18 cDNA in the expression plasmid Adori 2-12 B18 was driven by the cytomegalovirus(CMV) immediate early promoter and enhancer.

20

The Adori 2-12 vector was created by addition of an SV40 origin and enhancer to a known adenovirus vector (Barr et al., Gene Therapy 1:51 (1994); Davidson et al., Nature Genetics 3:219 (1993)). The HindIII/BamHI fragment encoding the SV40 origin and enhancer was isolated from the pMT2 mammalian expression vector, blunted with Klenow and cloned into the Natl site (blunted with Klenow) of the Ad5 expression vector.

25

30

The vector was derived by digesting pNOT-B18 cDNA with Sall, filling in the 5' overhang with Klenow to generate a blunt end and digesting with EcoRI to isolate the B18 cDNA. The blunted- EcoRI B18 fragment was inserted into the restriction sites EcoRV-EcoRI of the adenovirus vector Adori 2-12. The CMV-B18 expression cassette was located downstream of the SV40 origin and enhancer, and 0-1 map units of the left hand end of the adenovirus type 5(Ad5). The SV40 splice donor and

acceptor were located between the CMV promoter and B18 cDNA. Following the insert was SV40 poly A site, 9-16 map units of Ad5 and the puc 19 origin.

A recombinant adenovirus was generated by homologous recombination in 293 cells. AscI linearized Adori 2-12 B18 and ClaI digested AdCMVlacZ were introduced into the 293 cells using lipofectamine. Recombinant adenovirus virus was isolated and amplified on 293 cells. The virus was released from infected 293 cells by three cycles of freeze-thawing. The virus was further purified by two cesium chloride centrifugation gradients and dialyzed against PBS 4°C. Following dialysis of the virus glycerol was added to a concentration of 10 % and the virus was stored at - 70 °C until use. The virus was characterized by expression of the transgene, plaque forming units on 293 cells, particles/ml and Southern analysis of the virus.

5

10

15

20

25

30

A single dose of 5 X 10 ¹⁰ particles of recombinant adenovirus encoding B18 was injected into the tail vein of male C57/b16 mice, age 7-8 weeks. Control mice received an adenovirus encoding B-galactosidase. Four mice from each experimental group were killed on day 7 and 14. Blood was collected and automated hematologic analysis was performed using a Baker 9000. Differential counts were performed on blood smears. Tissue was harvested, fixed in formalin, and stained with hematoxylin and eosin for histopathology. In the first set of experiments, serum and tissues were analyzed 7 and 14 days post injection. A slight increase in peripheral platelet counts were observed. The animals that received B18 exhibited a slight increase in spleen size. Macroscopic analysis of the spleen showed an increase in splenic extramedullary hematopoiesis on day 7 compared to the control. These results showed a hematopoietic growth activity associated with B18.

In a second set of experiments 5 X 10 ¹⁰ particles of recombinant adenovirus encoding B18 were injected into the tail vein of male C57/bl6 mice, age 17-18 weeks. Control mice received an adenovirus encoding B-galactosidase. Blood samples were collected via retro-orbital sinus on days 2, 5, 7, 10, 14, and 21. The hematologic analyses were performed on the Baker 9000 automated cell counter with murine-specific settings. Analyses included WBC, RBC, HCT and PLT counts. Blood smears were prepared and stained with Wright-Geimsa for WBC differentials based on a 100 cellcount. Reticulocytes and reticulated platelets were quantitated using flow

5

10

15

20

25

30

cytometry. Four mice from each group were killed on days 7, 14, and 21. In addition to peripheral blood analysis, serum was collected via cardiac puncture for quantitation of systemic II-6 using a commercial kit (Endogen). Spleen and liver were collected for histopathology, spleen and bone marrow hematopoietic progenitors were quantitated, and bone marrow smears were prepared and stained with Wright-Geimsa for cell counts.

Administration of adenovirus encoding B18 resulted in a marked increase in peripheral blood neutrophils and WBC (Fig. 4). Maximum increases in neutrophils were observed at day 5 and day 7. The control mice showed, little difference at day 5 and day 7. Peripheral blood neutrophils were similar in the control mice and mice that received B18 at day 21. In both the B18 and control groups an increase in white blood cells was also observed. The mice that received B18 had a greater increase in WBC between day 2 and day 7. By Day 21 a more pronounced increase was observed in the B-gal group. No other changes in cellular chemistries were observed (Table I).

Bone marrow cellularity was calculated from pooled femurs in each group (Table III). No significant differences were observed in either group. No significant changes were observed in bone marrow hematopoietic progenitors from day 7, 14, and 21. The CFU-GM, BFU-E and CFU-MEG in the B18 mice were similar to the B-gal control (Table II).

Administration of the adenovirus encoding B18 resulted in an increase in CFU-GM (myeloid) and BFU-E (erythroid) progenitors in the spleen compared to animals that received the B-gal virus on day 7. The increase in progenitors in the B18 mice was 11-fold in CFU-GM and a 52-fold in BFU-E (Table II). There was a 2-fold increase in CFU-MEG at day 7 for the B18 mice. By day 21 no significant differences were observed in splenic CFU-MEG or BFU-E between the groups (Table II). A 3-fold decrease in CFU-GM was observed in mice that received adenovirus encoding B18. A slight increase in spleen size at day 7 was observed in the B18 group. This is consistent with an increase in splenic cellularity. By day 14 and day 21 spleen weights were similar to the control group (Table III). Macroscopic analysis of the spleen showed an increase in splenic extramedullary hematopoiesis of the B18 mice on day 7 compared to the control.

5

1Û

The bone marrow myeloid: erythroid ratios (Table IV) suggest a granulocytic hyperplasia with a possible erythroid hypoplasia in mice that received adenovirus B18 on day 7. By day 21 the ratio in the B-gal group was higher. No changes were observed in the IL6 serum levels.

These results show a hematopoietic activity associated with the administration of adenovirus encoding B18 (human CTLA-8). Increases in neutrophils and white blood cells were observed at day 7 in animals that received B18 adenovirus. The data showed that B18 resulted in increase in splenic CFU-GM and BFU-E 7 days post administration compared to the control animals. Splenic extramedullary hematopoiesis on day 7 support that B18 exhibits a hematopoietic growth activity. These data suggest that B18 may mobilize early hematopoietic precursors.

Table I: Peripheral hematology for day 2, 5, 7, 10, 14, and 21.

Group A	BIB (Plate	lets)	Day 24-2	25-98.											
Croup A	WBC	Neuts	ANC	Lymphs	ALC	Eas	Monos	RBC	Retics	Abs Retics	HCT	PLT	RP#	Abs RP8	
B-Gal #1	x10^3/ut.	- %	x10^3/uL	34	2.92 2.92	*	<u>*</u>	rtoኅደሌሲ	<u>*</u>	#10^6A.L	×	×10^3ArL	×	x10 43A.L	
9-G# #2	7.4	40 25	2,16 1,85	65	2,92 4 ft	0	8 7	10,88	3.65 2.04	0.40 0.25	48.0	835	11,94	99.82	
B-Gal #3	6.6	23 40	2.72	52	3.54	3	6	11.25	3.26	0.25	56.6 51.6	900 894	10.10 9.77	90.90 87,34	
B-Gat #4	8.8	23	2.02	64	5.63	î	12	12.00	2.55	0.37	54.8	840	10.63	87.34 89.29	
AVG	7.1	32.0	2.19	58.8	4.22	1.5	7.6	11.52	2.11	623	\$2.5	958	15.61	ىدە	
SEM	0.7	4.6	0.19	3,4	0.51	0.6	1.4	0.33	0.36	0.03	1.5	17	0.48	2.75	
B(8 et	11.4	59	6.73	31	3.53	-		11.16	4.88	0.54	52.4	1242	14.92	185.31	
818 42	9.2	30	2.76	62	5.70	3	5	10.14	3.97	0.40	48.0	632	10.90	68.89	
Ø10 e3	5.0	51	2.55	40	2.00	0		11,15	323	0.36	51.2	632	11.18	83.02	
818 #4	6.4	41,	2.52	55 47.0	3.52	0,_	4	10.60	3.09	0.33	49.2	904	17.31	156.48	
AVG SEM	8.0	45.3	3.67 1.02	47.0 7.0	9.69	1.0	13	10.52	3.79	M1	50.2	903	12,54	125.92	
SEM	1.4	6.3	1,02		0.76	- 0.7		0.24	0.41	0.05	1.0	127	1.55	27.07	
Study AS4-1B.	B18 (Platel	lets)	Day 5_4-2	20-06,											
Group B	WBC	Neuts	ANC	Lymphs	ALC:	Eos	Monos	Rec	Retics	Abs Retics	ИСТ	PLY	RPti	Abs RPs	
	21043AL	× ·	A10-3VT	*	110 43AL	- *	_ % .	X10"ENL	*	X1046AA	*	#10*3A/L	**	AVE- OLK	
B-Gal #1	7.6	14	126	78	5.93	3	5	11.26	5.25	0.59	524	1082	15.51	167,82	
6-G= 12	10,6	20	2.12	76	6.27	1	,:1	1073	4.71	0.50	49.4	.994	17.37	172,66	
B-Q# 43	8.6	18	1.51	69	6.07	2	31	11,12	3.40	9.23 j	ői£	916	9.65	87.48	
Brun ii	10.5	₩	4.10	56	6.26	9	4	10.22	6.21	0.63	47.0	1092	13.93	152.12	
AVG	8.5	72.5 5.3	2.20 0.57	79.J	6.63 0.55	1.5 0.6	5.3	10.53	4.89	653	50.0	1021	14.09	145.02	
\$EM	14.5	18	2.66	71	10.51		10 2.1	12.56	2.31	0.06	12	41	1,57	19.67	
818 62	142	37	525	53	7.53	2		9.80	3.32	0.29	67.0	1204	7.57	91.14	
B18 #3	12.5	só.	3.64	59 59	7.55	i '	10	12.12	4.12	0.33 0.50	44.6 53.8	888 1134	14.33	127.25 115.10	
B18 #4	18.0	58	9.25	37	592	6	s :	11.04	3.03	943	50.8	1156	10.15	183.65	
AVG	14.5	~35.a	5.25	\$5.0	7,88	Ĭ 1.0	اده	1141	3.42		5U.5	100	11.05	123.24	
SEM	0.7	4.4	1,44	- /3	0.96	0.4	12	0.63	0.41	8.05	2.8		1.55	19.61	
														15-41	
Study AS4-(B_	B18 (Platel	eta)	Day 7, A	0-96.											
Guorib C	WEC	House	ANC	Lymphs	ALC	Eco	Monos	RBC	Notice	Abs Retics	HCT	PLY	RPtt	Abe RPs	
B-Call at	x10*3/uL	- 12 -	210*3AL	69	10.4341	<u>*</u>	16	21046AL	2.54	*1046AL	<u> %</u>	X1003AL	<u>*</u>	x10 *3Ad.	
B-Gal #2	140	12	1.68	81	11.34	,	7 1	11.30	3.54 8.05	0.29	50.1 52.6	862	12.45	107.A1	
B-Gal es	14.8	14	207	73	10.60	. i	i i	10.02	5.42	0.57	49.5	1104	14.01	194.61	
AVG	147	122	1.06	74.3	18.62	0.7	117	11.11	4.67	9.52	61.0	1002 1773	12.95	127.13	
SEM	0.4	0.7	0.14	3.5	0,25	6.5	2.5	0.14	0.58	0.04	0.0	"41	1.02	18.75	
B18 #1	184	33	6.40	62	12.03	0	3	10.14	2.93	030	452	864	12.00	110.59	
B18 #2	25.4	39	9.91	53	13.46	Ó	8	9.48	6.05	0.57	43.5	1258	12.49	180.87	
B18 #3	23.6	44	10.39	50	11.80	0	0	9.74	£.17	0.50 Ì	44.4	1076	15.41	165.81	
B18 64	12.8	15	1.92	75	1.60	0	. 10	0.54	6.26 -	0.60	43.4	1136	15.88	180.40	
AVG	507	22.3	7.15	60.0	11.72	9.0	7.3	8,72	8.10	6.43	44.2	1091	14.15	154.42	
M33	2.1		1.96		<u> </u>	00	1.1	0,15	0.78	0.07	0.4	81	0.87	15.19	
Study AS4-4B_	B18 (Platel														
			Day 10 R	3.04											
Group A		(ets)	Day 10_5 ANC		ALC T	Eos	Monoa	REC 1	Better	N-Bales (Det 1	- 10 7 1	50w	Abo bibs	
Group A	₹18¢ 10^3A±L		ユマドゲト	Lympha %.	ALC MARY BIN	Eos X	Monos	RBC x10^6AL	Retice %	Abs Retics	HET	- PLT	APR %	Abe RPR	
Group A	184 184 184	Houses 94 17	#10^2/sL 3.16	Lymphs %.	12.83	¥ .	Monos %	1022	1241	Also Helica x1046Ad.	HCT VA	110^3AL	_ %	210 -3A4.	
Group A B-Gal #1 B-Gal #2	186 10^3AsL 18.6 13.2	Hecits 17 10	ANG #10^20/sL 3.16 2.11	Lymphs 69 79	12.83 10.43	3	11	10-644 10-22 10-48	12.41 6.00	127 0.63	46.8 48.8	110^3/4 1460 1129	18.20 14.48	236.52 163.33	
Group A B-GM #1 B-GM #2 B-GM #3	186 186 132 198	17 10 16	ANC #10*254L 3.16 2.11 2.14	69 79 79	12.63 10.43 14.50	3	17 4 10	10.22 10.48 10.72	12.41 8.00 6.25	127 0.63 0.67	46.6 46.6 46.6	110^3A4 1460 1120 1338	18.20 14.48 18.58	276.52 163.23 221.84	
B-GM #1 B-GM #2 B-GM #2 B-GM #4	185 10^3/d 186 132 198 186	17 18 18 18 21	ANG #10*254 2.16 2.11 2.14 2.91	69 79 74 72	10.43 10.43 14.50 11.39	* ·	17 4 10	10-22 10-22 10-48 10-72 10-44	% 12.41 6.00 6.25 7.59	1.27 0.63 0.67 0.79	46.8 46.8 49.4 49.4	1460 1460 1129 1336 1068	18.20 14.48 18.58 14.35	236.52 163.23 221.84 153.25	
Group A B-GM 61 B-GM 82 B-GM 63 B-GM 64 AVG	185 10-344 186 132 196 185 175	17 10 16	ANC #10*254 3.16 2.11 2.14 2.91 3.08	69 79 74 72 72.5	18 *2 & L 12 & L 10 & A S 14 & S 12 &	¥	11 4 10 4 7.5	210^6A4 10.22 10.48 10.72 10.44 10.47	92 12.41 8.00 6.25 7.59	210-EAE. 1.27 0.63 0.67 0.79	45 45 45 45 45 45 45 45 45 45 45 45 45 4	110^3A4 1460 1120 1338	16.20 14.48 18.58 14.35 15.40	200.52 163.25 271.84 153.26 183.74	
B-GM #1 B-GM #2 B-GM #2 B-GM #4	185 10^3/d 186 132 198 186	17 18 18 18 21	ANG #10*254 2.16 2.11 2.14 2.91	69 79 74 72	10.43 10.43 14.50 11.39	* ·	17 4 10	10-22 10-48 10-72 10-44 10-47 9,10	% 12.41 8.00 6.25 7.59 8.06	210-644. 1.27 0.63 0.67 0.79 8.84 0.15	46.5 46.5 49.4 49.4 49.4 49.4 68.	110^3A4 1460 1129 1338 1068 1249	18.20 14.48 18.58 14.35 15.40 8.58	270 -3/4. 270.52 163.23 221.84 153.26 183.74 20.78	
B-GM 61 B-GM 62 B-GM 63 B-GM 64 AVG SEM B18 61	186 10-3/4 186 132 198 188 17.5 1.5	Hauts % 17 10 16 21 17.5 1.2	AHC #10*254 3.16 2.11 2.14 3.91 3.08 0.37	69 79 74 72 73.5 2.1	10 *3/4L 12.83 - 10.43 14.50 12.39 12.79 0.88	¥	11 4 10 4 7.5	10-22 10-22 10-48 10-72 10-44 10-47 0-10	% 12.41 8.00 6.25 7.59 8.06 1.49	x10^4AL 1,27 0,63 0,67 0,79 0,44 0,15	22 463 464 464 464 464 464 464 464 464 464	110^3A4 1460 1129 1336 1068 1249 91	16.20 14.48 18.68 14.35 15.40 8.58	201.25 153.25 153.25 153.26 153.26 153.26 153.26 263.71	
B-Gal #1 B-Gal #2 B-Gal #2 B-Gal #4 AVG SEM B18 #1 B18 #2 B18 #2	1186 1003Ad 168 132 198 188 17.5 1.5 142	17 18 16 16 21 17.5 1.2 33 35	210 211 211 214 291 381 489 488 697	59 79 74 72 73.5 2.1 56 57	10.43 10.43 14.50 12.39 12.79 0.65 7.95 10.00	1.9 2 1.9 0.8	7.5 1.9	10-22 10-48 10-72 10-44 10-47 9,10	% 12.41 8.00 6.25 7.59 8.06 1.49 11.97 8.48	\$10^6AL 1.27 0.63 0.67 0.79 8.84 0.15	46.5 46.5 49.4 49.4 49.4 49.4 68.	110^3A4 1460 1129 1338 1068 1249	16.20 14.48 16.68 14.35 15.40 6.58 14.49 18.88	206.52 163.23 221.84 153.26 153.26 183.74 20.78 255.02 208.44	
B-GM #1 B-GM #2 B-GM #2 B-GM #4 AVG SEM B18 #1 B18 #2 B18 #3 B18 #4	1186 1003Ad 168 132 198 188 17.5 1.5 142 17.8 162 142	17 10 16 16 21 17.5 1.2 33 35 39 25	210 110 211 211 214 291 291 483 483 483 483	59 79 74 72 73.5 2.1 56 57 57	10 *2kd 12.83 - 10.43 14.50 12.39 12.79 0.88 7.05 10.00 9.23	1.9	7.3 1.9 1.9	#10^6A.L 10.22 10.48 10.72 10.44 10.47 8,10 E.70	12.41 8.00 6.25 7.69 8.06 1.45 11.97 2.48 16.77 8.90	x10^4AL 1,27 0,63 0,67 0,79 0,44 0,15	46.8 46.8 49.4 49.4 48.4 6.8 39.7 42.0	1460 1129 1338 1068 1249 1760 1104 894	16.20 14.48 16.58 14.33 15.40 8.58 14.49 18.88 89,10	206.52 163.23 163.26 153.26 153.26 153.76 20.78 255.02 256.44 260.96	
B-GM #1 B-GM #2 B-GM #2 B-GM #3 B-GM #4 AVG B18 #1 B18 #2 B18 #3 B18 #4 AVG	1186 1109Ad 118 112 118 118 118 118 118 118 118 118	17 18 16 16 21 17.5 1.2 33 35	210 211 211 214 291 381 489 488 697	59 79 74 72 73.5 2.1 56 57	10 *2kd 12.83 10.43 14.50 12.39 12.79 0.48 7.45 10.00 9.23 8.37	2 14 03 5 ·	7.3 10 4 7.3 1.9	10-22 10-49 10-72 10-44 10-47 0-10 8-70 8-74 4-74 13-30 7-85	% 12.41 6.00 6.25 7.59 8.06 1.57 2.48 16.77 2.40	210-444. 127 0.63 0.67 0.79 8.84 0.15 1.04 0.86 0.79 0.92	48 48 44 4 4 8 8 8 8 8 8 8 8 8 8 8 8 8	1160 1160 1128 1338 1068 1249 91 1760 1104	16.20 14.48 16.68 14.35 15.40 6.58 14.49 18.88	206.52 163.23 221.84 153.26 153.26 183.74 20.78 255.02 206.44	
B-GM #1 B-GM #2 B-GM #2 B-GM #4 AVG SEM B18 #1 B18 #2 B18 #3 B18 #4	1186 1003Ad 168 132 198 188 17.5 1.5 142 17.8 162 142	17 10 16 16 21 17.5 1.2 33 35 39 25	210 110 211 211 214 291 291 483 483 483 483	59 79 74 72 73.5 2.1 56 57 57	10 *2kd 12.83 - 10.43 14.50 12.39 12.79 0.88 7.05 10.00 9.23	1.9	7.3 10 4 7.3 1.9	10-22 10-49 10-72 10-44 10-47 10-47 10-47 10-47 10-47 10-47 10-47 10-47 10-47 10-47 10-47 10-47	12.41 8.00 6.25 7.69 8.06 1.45 11.97 2.48 16.77 8.90	210^4AL 1.27 0.63 0.67 0.79 0.44 0.15 1.04 0.66 0.79 0.62	44444 4444 3000 3000 3000 3000 3000 300	110°3A4 1460 1129 1338 1069 1249 91 1760 1104 894 1418	16.20 14.48 16.58 14.35 15.40 6.58 14.49 18.89 18.89	236.52 183.33 183.25 183.25 183.26 183.74 20.78 255.02 206.44 260.95 226.03	
Group A B-GM #1 B-GM #2 B-GM #3 B-GM #4 AVG B18 #3 B18 #3 B18 #3 AVG SEM	118C 1003Ad 188 132 198 188 17.5 148 17.5 142 17.8 18.2 14.2 18.6 0.8	Nexts % 17 18 16 21 17.5 1.2 33 35 39 25 -33.0 2.9	ANC #10*25/L 2.15 2.11 2.14 2.91 3.05 0.97 4.69 8.18 6.72 2.55 8.18 6.72 2.55	57 57 58 89.0 2.3	10 *2kd 12.83 10.43 14.50 12.39 12.79 0.48 7.45 10.00 9.23 8.37	1.9	7.3 10 4 7.3 1.9	10-22 10-49 10-72 10-44 10-47 0-10 8-70 8-74 4-74 13-30 7-85	% 12.41 6.00 6.25 7.59 8.06 1.57 2.48 16.77 2.40	210-444. 127 0.63 0.67 0.79 8.84 0.15 1.04 0.86 0.79 0.92	48 48 44 4 4 8 8 8 8 8 8 8 8 8 8 8 8 8	110^3Ad 1460 1129 1338 1068 1249 91 1760 1104 894 1418 1254	16.20 14.46 18.56 14.35 15.40 8.58 14.49 18.60 29.10 18.61	206.52 163.53 221.84 153.26 183.74 263.72 264.44 260.96 226.73 240.81	
Group A B-GM 61 B-GM 62 B-GM 63 B-GM 64 AVG B18 61 B18 62 B18 64 AVG SEM SEM SEM	1186 110-3Aul 188 112 198 188 17.5 14.2 17.8 16.2 14.2 15.6 0.8	Hearts % 17 18 16 21 17.5 1.2 33 35 39 25	ANC #10*251 3.16 2.11 2.14 2.91 3.06 0.37 4.59 8.18 8.72 3.55 8.72 3.55 8.72 3.55 8.72	50 57 56 89.0 2.3	12.61 12.61 10.43 14.50 13.39 12.79 0.66 7.95 10.00 6.20 8.37 8.15 0.41	1.0 2 1.0 0.0 1.0 1.0	7.5 1.9 1.9 1.9 2.5 2.5 2.5 2.5	710^6A.L. 10.22 10.48 10.77 10.44 10.47 8.10 8.70 8.04 4.74 13.30 7.85 1.01	% 12.41 8.00 6.25 7.69 8.06 1.45 11.97 2.46 18.77 8.90 12.04 1.57	210-644 127 0.53 0.67 0.79 0.15 1.04 0.66 0.79 0.92 0.90	46.5 48.6 48.4 48.4 48.4 68 58.2 60 52.1 60 52.1 60 52.1 60 50 50 50 50 50 50 50 50 50 50 50 50 50	110^3A4 1460 1129 1338 1068 1249 91 1760 1104 894 1418 1254 119	15.20 14.48 16.55 14.35 15.40 6.58 14.49 18.83 25,10 18.81 19.84 3.24	210 *3/4. 236.52 165.33 221.84 153.26 183.74 20.78 255.02 206.44 260.95 240.81 11.77	
Group A B-GM #1 B-GM #2 B-GM #3 B-GM #4 AVG B18 #3 B18 #3 B18 #3 AVG SEM	118C 119-3Asl. 18.6 13.2 19.6 18.5 15.5 14.2 17.8 18.2 14.2 17.8 18.2 14.2 18.6 0.4	Nexts % 17 18 16 21 17.5 1.2 33 35 39 25 -33.0 2.9	ANC H0424 3.16 2.11 2.14 2.91 3.08 0.17 4.69 8.18 8.18 8.19 8.19 8.19 8.19 8.19 8.1	57 57 58 89.0 2.3	10 *3AL 12.53 - 10.43 - 14.50 12.79 0.48 7.05 10.00 9.23 9.37 8.37 8.15 0.43	1.9	7.3 10 4 7.3 1.9	10.22 10.22 10.49 10.72 10.44 10.47 9.10 8.70 8.04 4.74 10.30 7.85 1.08	% 12.41 6.00 6.25 7.59 8.06 1.57 2.48 16.77 2.40	210-644. 127 0.53 0.67 0.79 0.44 0.15 1.04 0.86 0.79 0.90 0.90 0.90 0.90 0.85	48 48 44 4 4 8 8 8 8 8 8 8 8 8 8 8 8 8	110^3/Ac 1460 1129 1338 1068 1249 91 1760 1104 894 1418 1254 119	16.20 14.46 18.56 14.35 15.40 8.58 14.49 18.60 29.10 18.61	210 *3A4, 236,52 161,53 121,84 151,26 183,74 203,78 255,02 206,44 260,96 260,96 260,96 240,81 11,177	
Group A B-GM 61 B-GM 62 B-GM 63 B-GM 64 AVG B18 61 B18 62 B18 64 AVG SEM SEM SEM	118C 10-3Ad 18.6 13.2 19.8 18.6 17.5 14.2 17.8 16.2 16.2 16.2 16.2 16.2 16.3 16.2 16.3 16.2 16.3 16.3 16.3 16.3 16.3 16.3 16.3 16.3	Hearts % 17 18 16 21 17.5 1.2 33 35 39 25	ANC #10*251 3.16 2.11 2.14 2.91 3.06 0.37 4.59 8.18 8.72 3.55 8.72 3.55 8.72 3.55 8.72	50 57 56 89.0 2.3	12.61 12.61 10.43 14.50 13.39 12.79 0.66 7.95 10.00 6.20 8.37 8.15 0.41	14 03 14 15 15 15 15 15 15 15 15 15 15 15 15 15	7.5 10 4 7.5 7.5 7 3 8 6.0 1.1	710^6A.L. 10.22 10.48 10.77 10.44 10.47 8.10 8.70 8.04 4.74 13.30 7.85 1.01	% 12.41 8.00 6.25 7.59 8.06 1.49 11.57 8.48 18.77 8.90 12.04 1.57	#10**EAU. 1.27 0.653 0.87 0.79 8.84 0.15 1.04 0.86 0.79 0.92 0.90 0.85	46.5 48.6 48.4 48.4 48.4 68 58.2 62.0 52.1 62.0 52.1 62.0 54.4 4.7	110^3/AL 1460 1129 1338 1068 1249 91 1760 1104 894 1418 2254 119	15,20 14,48 16,58 14,35 15,40 6,58 14,49 18,83 29,10 18,81 19,84 3,24 RPR	210 ** 3Ad. 205.52 165.33 221.84 155.26 185.74 205.84 260.96 240.81 11.77 Abs. RPR. 210 ** 3Ad.	
Group A Bidal #1 Bidal #2 Bidal #3 Bidal #4 AVG Bite #1 Bite #2 Bite #2 Bite #2 Bite #4 AVG SEM State #4 Group B Bidal #1 Bidal #1 Bidal #1 Bidal #1	1186 119-344 148 132 188 188 178 142 178 182 142 142 188 188 188 188 188 188 188 188 188 18	Hences % 10 16 16 12 17.5 1.2 33 35 39 25 39,9 6tr) Hences %	ANG MIONANA MIONANA ANG ANG ANG ANG ANG ANG ANG	1, prophs	10 43 ALC 12 Bi 10 AI 10 AI 10 AI 12 Di 12 Di 12 Di 12 Di 12 Di 12 Di 13 Di 14 Di 15 AI 16 AI 16 AI 16 AI 17 AI 18 AI	14 0.3 1.4 0.5 1.0 1.0 1.0 1.0 1.0 1.0 1.0 1.0 1.0 1.0	75 119 10 4 7.5 1.9 8 8.0 1.1 1.1 1.1 1.1 1.1 1.1 1.1 1.1 1.1	#10*66.4 10.22 10.42 10.47 10.47 0.10 E/O E/O E/O E/O E/O E/O E/O E/O E/O E/O	% 12.41 8.00 8.23 7.59 8.06 1.49 11.97 8.48 18.77 8.83 12.04 1.57 Redica %	107-644 127 0.63 0.67 0.79 0.44 0.65 0.79 0.92 0.92 0.95 Abe Retice pro-644	46.5 46.5 46.4 46.4 46.4 46.4 46.4 46.4	110^3/Ac 1460 1129 1338 1068 1249 91 1760 1104 894 1418 1254 119	15.20 14.48 16.58 14.35 15.40 6.58 14.49 18.89 29.10 18.81 19.44 3.24	200-52 183.33 221.84 183.34 20.72 205.02 206.44 200.96 240.61 11.77 Abs RPR xtd «3.04 150.01	
Group A B-Gal #1 B-Gal #2 B-Gal #2 B-Gal #4 AVG B16 #2 B16 #2 B18 #3 B18 #3 B18 #4 Group B B-Gal #1 B-Gal #2 B-Gal #2	1185 10-344 188 188 132 188 188 188 142 178 182 142 158 08 818 (Pute WBC vt 178 204 178	Hences 4, 17 18 16 16 21 17.5 33 35 35 39 25 30.0 10 10 29	ANC HI0^2Md 2.15 2.11 2.11 2.91 3.93 4.69 4.18 6.18 6.18 6.18 6.19 4.60 4.60 4.60 4.60 4.60 4.60 4.60 4.60	1, proprie %. 95. 97. 77. 77. 77. 56. 57. 66. 69.0 7.9 74. 66. 69.0 90.0 90.0 90.0 90.0 90.0 90.0	10.43 (10	13 13 13 13 13 13 13 13 13 13 13 13 13 1	7.5 10 4 7.5 7.5 7 3 8 6.0 1.1	#10^6/d. 10.22 10.42 10.44 10.47 10.44 10.47 0.10 E.70 E.70 E.74 8.30 7.85 1.61 10.56 10.56 10.56 10.56	% 12.41 12.00 6.25 7.59 9.06 1.49 11.57 2.48 16.77 8.90 12.04 1.57	#10**EAU. 1.27 0.653 0.87 0.79 8.84 0.15 1.04 0.86 0.79 0.92 0.90 0.85	44.4.4.4.4.4.4.4.4.4.4.4.4.4.4.4.4.4.4	100-9Az 1460 1129 1338 1068 1249 1760 1104 834 1418 1254 189 FLT \$10-34c 1360	15,20 14,48 16,58 14,35 15,40 6,58 14,49 18,83 29,10 18,81 19,84 3,24 RPR	210 *3Ad, 226.52 165.23 221.84 153.26 183.74 205.44 260.65 240.61 11.37 Abs RPR x10 *3Ad, 150.01 150.01	
Group A Bidla 61 Bidla 62 Bidla 63	186 186 186 186 188 188 188 188 188 182 162 162 162 162 162 162 162 163 164 164 164 165 166 166 166 166 166 166 166 166 166	Henrica	ANC MONTH ANC MONTH AND ANC AND	1, prophs	12.63 10.43 14.50 12.79 0.66 7.95 10.00 9.23 9.37 14.50 13.17 13.46 14.66 110.25	50 2.0 1.0 1.0 1.0 1.0 1.0 1.0 1.0 1.0 1.0 1	% 1.9 1.9 1.9 1.19 Monos % 7 2 8 6.0 1.1	#10°604 10.22 10.42 10.47 10.47 0.10 E.70 E.70 E.70 E.74 2.30 1.08 #86CV 10.56 10.92 11.28	% 1221 8.00 6.25 7.59 8.06 11.97 8.46 16.97 12.04 1.57 Retica % 8.97 7.07 6.41	#10-6Ad. 1.27 0.63 0.87 0.79 0.94 0.46 0.79 0.92 0.90 0.05 Abe Redice	46.8 48.8 48.4 48.4 6.8 58.2 6.8 58.2 6.8 58.2 6.8 58.2 6.8 58.2 6.8 58.2 6.8 58.2 6.8 58.2 6.8 58.2 6.8 58.2 6.8 58.2 6.8 58.2 6.8 58.2 6.8 58.2 6.8 58.2 6.8 58.2 6.8 58.2 6.8 58.2 58.2 58.2 58.2 58.2 58.2 58.2 58	10~3/4c 1460 1129 1129 11398 1058 1058 1058 11760	15.20 14.48 14.45 14.45 15.40 15.40 16.85 19.10 18.81 19.84 3.24 11.03 8.18 7.36	#10 **Ad. 200.52 183.24 183.24 183.24 183.26 183.27 200.44 200.96 240.81 11.77 Abs RPR #10 **2ad. 150.07 152.19 85.53	
Group A B-Gall #1 B-Gall #2 B-Gall #2 B-Gall #4 AVG B18 #2 B18 #3 B18 #3 B18 #4 AVG SEM Such AVG Group B B-Gall #1 B-Gall #1 B-Gall #2 B-Gall #3 B-Gall #3 B-Gall #3 B-Gall #3	186 (1966) 187 187 187 187 187 187 187 187 187 187	Hencts K: 17 10 16 21 17.5 32 39 25 39 25 10 10 10 10 10 10 10 10 10 10 10 10 10	ANG MID-SAME ANG MID-SAME ANG MID-SAME ANG	Lymphs 5. 5. 7. 7. 7. 5. 5. 7. 5. 7. 5. 7. 5. 7. 5. 7. 5. 7. 5. 7. 5. 7. 5. 7. 5. 7. 5. 7. 5. 7. 5. 7. 5. 7. 5. 7. 5. 7. 5. 7. 7. 5. 6. 90 57 7.1.2	#10 **2 Art. 12.53 10.43 14.50 12.39 12.79 0.45 12.79 0.45 10.00 6.23 8.37 8.15 6.43 13.47 13.46 14.40 10.26 12.22	5 1 2.0 5 1 1 1 0.0 5 1 1 1 0.0 5 1 1 1 1 0.0 5 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	75 119 10 4 7.5 1.9 8 8.0 1.1 1.1 1.1 1.1 1.1 1.1 1.1 1.1 1.1	#10-66-16 10-22 10-48 10-72 10-44 10-47 10-44 10-47 10-64 10-64 10-65 10-66 10	% 12.41 12.41 12.60 12.50 12.50 12.50 13.50	10-644. 127 0.53 0.67 0.79 0.94 0.15 1.04 0.86 0.79 0.92 0.90 0.05 Ape Redes p10-641. 0.85 0.77 0.73 0.73 0.73 0.73	**************************************	10-3/4c 1460 1120 1120 1120 1238 1088 1088 11760 11760 11760 1418 1254 1199 PLT 510-3/4c 1360 1616	15.20 14.48 14.53 14.43 14.53 14.53 14.49 18.68 123.19 18.81 123.14 3.24 11.03 8.16 10.03 8.16	200-244, 200-25 (2014)	
Group A B-Gal #1 B-Gal #2 B-Gal #3 AVG SEM B18 #2 S18 #3 S18 #3 SEM AVG AVG AVG AVG AVG AVG AVG AV	186 (196) 186 1	Hereta	ANG 110-20-4 2-11 2-1	1,700 to 1,7	10-73-64 10-43 10-43 14-50 12-29 0.45 7-95 10-20 8-37 8-15 0.43 ALC KB *33-64 13-17 13-16 14-40 10-26 12	50 2.0 1.0 1.0 1.0 1.0 1.0 1.0 1.0 1.0 1.0 1	7.3 1.9 1.5 1.5 1.5 1.5 1.5 1.5 1.5 1.5 1.5 1.5	#10*6% 10.42 10.42 10.42 10.47 0.10 E.70 E.70 E.74 4.74 8.30 7.85 1.08 10.85 1	% 12.41 E.00 E.25 7.59 E.06 11.97 2.48 11.97 2.90 12.04 1.57 7.07 E.87 7.07 E.41 7.52 E.41 7.52 E.41 7.52	10-644. 127 0.53 0.87 0.99 0.45 0.15 0.09 0.86 0.79 0.90 0.85 409 0.05 409	468 488 484 484 68 822 820 824 47 47 808 808 828 420 421 421 421 421 421 421 421 421 421 421	TIDASALE TIDASALE TIDAS	15.20 14.48 14.48 14.43 15.40 6.58 14.43 18.88 19.10 18.81 19.81 19.81 19.81 11.03 6.16 7.36 11.03 6.16 7.36	20-24 20-52 20-52 20-52 20-54 153.26 153.26 153.26 20-52 20-54 20-56 20-56 20-56 150.7	
Group A B-Gall #1 B-Gall #2 B-Gall #2 B-Gall #4 AVG B18 #2 B18 #3 B18 #3 B18 #4 AVG SEM Sbuty AS-4B B-Gall #1 B-Gall #2 B-Gall #3	18.6 12.2 18.6 18.6 17.2 18.5 18.5 17.5 18.2 17.5 18.2 14.2 17.6 18.2 14.2 17.6 18.0 18.0 18.0 18.0 18.0 18.0 18.0 18.0	Hearts K: 17 10 16 21 17-5 12 33 35 39 25 33.0 23 Nexts Nexts 10 20 7 35 21,8 9,1	ANG #10-20-4 2-14 2-14 2-14 2-15 2-16 2-17 2-18 2-1	Lymphs 5	#10 **2Ard. 12.251 10.452 14.50 12.359 12.379 6.451 10.000 6.257 8.15 6.431 13.17 13.46 13.17 13.46 13.17 13.46 13.47 13.48 13.49 13.25 12.25 0.25 12.25 0.25 12.25 0.25 12.47 12.25 0.25 0.25 0.	2.0 2 1.4 2.5 5 1 1 1.0 5 0 1	50 1.9 1.9 1.0 1.1 1.9 1.1 1.9 1.1 1.9 1.1 1.1 1.1 1.1	#10-66-14 10-22 10-49 10-72 10-49 10-72 10-44 10-47 10-67 10-68 10-68 10-68 10-68 10-68 10-68 10-68 10-68 10-68 10-68	% 12.41 8.00 6.25 7.59 8.06 11.57 8.49 11.57 8.97 8.97 8.97 8.97 8.97 8.97 8.41 7.62 8.77 8.35 8.77	#10-64d. 127	46.6 48.6 48.4 48.4 68.5 82.5 82.6 82.6 82.6 82.6 82.6 82.6 83.6 83.6 83.6 83.6 83.6 83.6 83.6 83	TIO-3A4. 1460 1129 1328 1028 1029 11760 1106 894 1418 1254 1199 PLT \$TO-3A4. 1298 1676 1298 1676 1298 1677 1447 1298 1677 1298 1677 1447 1298 1677 1298 1677 1298 1677 1298 1677 1298 1677 1298 1677 1298 1677 1298 1677 1298	15.20 14.48 14.53 14.43 14.33 14.43 14.33 14.49 14.29 14.29 14.24 3.24 11.23 6.16 7.36 10.25 8.16 0.24	170 - 2444 20132 20132 20134 151,267 151,267 20144 2005 240,51 111,77 112,19 120,10 122,19 130,01 130,04	
Group A B-GM #1 B-GM #1 B-GM #2 B-GM #4 AVG B18 #2 B18 #2 B18 #3 AVG SEM AVG SEM AVG SEM AVG SEM AVG SEM B-GM #1 AVG B-GM #1 B-GM #2 B-GM #4 AVG B-GM #4 B-GM #2 B-GM #4 B-GM #2 B-GM #4	186 (196) 186 1	Hearts H	ANG 110-20-4 2-11 2-1	1,700 to 1,7	#18 **2AAL 12.53 1 10.43 14.50 12.39 12.79 6.23 6.43 13.17 6.43 13	5 1 2.0 5 1 1 1 0.0 5 1 1 1 0.0 5 1 1 1 1 0.0 5 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	14 10 1.9 1.9 1.1 Monos 1.1 1.1 1.1 1.1 1.1 1.1 1.1 1.1 1.1 1.	#10-6A4 10.22 10.43 10.47 0.10 E.70 E.70 E.70 E.70 E.70 E.70 E.70 E.7	% 12.41 £.00 £.25 7.59 £.06 1.49 11.57 £.46 16.77 £.90 12.04 1.57 6.41 7.07 6.41 7.02 6.41 7.03 8.77	#10-64.d. UE7 0.85 0.87 0.87 0.15 1.04 0.15 1.05 0.50 0.70 0.05 0.77 0.77 0.77 0.77 0	46.6 48.8 48.4 48.4 48.4 48.4 48.4 53.2 53.2 52.8 52.8 52.8 53.8 53.8 53.8 53.8 53.8 53.8 53.8 53	TIO-3/LC 1460 1129 1329 1329 1068 1249 1760 1104 894 1418 1294 1418 1294 1360 1616 1293 1672 1487 1293 1672	15.20 14.48 14.48 14.53 14.49 14.53 14.49 14.69 18.81 19.84 3.24 11.03 8.10 7.30 8.10 7.30 8.10 7.30 8.10 19.81 19	100-044 200-22 200-22 201-24 101-28 201-44 201-26 201-2	
Group A B-Gall #1 B-Gall #2 B-Gall #2 B-Gall #4 AVG B18 #2 B18 #3 B18 #3 B18 #4 AVG SEM Sbuty AS-4B B-Gall #1 B-Gall #2 B-Gall #3	1186 110-344 188 1132 188 188 17.5 18.2 17.8 18.2 14.2 17.8 18.2 14.2 17.8 18.2 18.6 17.6 20.4 18.0 18.0 18.0 18.0 18.0 18.0 18.0 18.0	Hearts K: 17 10 16 21 17-5 12 33 35 39 25 33.0 23 Nexts Nexts 10 20 7 35 21,8 9,1	450 410 410 410 410 410 410 410 410 410 41	Lymphs 55. 69 79 74 72 71.55 2.1 560 82.0 2.1 74 66 80 90 57 71.8 75 66 80 90 57 71.8 75 66 80 90 57 71.8 75 66 80 90 57 71.8 75 66 80 90 57 75.8 80 90 57 75.8 80 90 57 75.8 80 90 57 75.8 80 90 57 75.8 80 90 57 75.8 80 90 57 75.8 80 90 57 75.8 80 90 57 75.8 80 90 57 75.8 80 90 57 75.8 80 90 57 75.8 80 90 57 75.8 80 90 57 75.8 80 90 90 90 90 90 90 90 90 90 90 90 90 90	#18 **2AAL 12.53 1 10.43 14.50 12.39 12.79 6.23 6.43 13.17 6.43 13	20 1.0 1.0 1.0 1.0 1.0 1.0 1.0 1.0 1.0 1.	50 1.9 1.9 1.0 1.1 1.9 1.1 1.9 1.1 1.9 1.1 1.1 1.1 1.1	#19-644 10.22 10.24 10.24 10.72 10.44 10.17 6.10 8.70 8.70 8.70 10.85 10	% 12A1	#10-cent. 127 0.87 0.87 0.84 0.15 0.90 0.	448 444 444 444 45 582 200 364 47 47 482 483	TIO-SALE TIO-SALE TIO-SALE TIOS TIOS TOS TOS TOS TOS TOS TOS TOS TOS TOS T	16.20 16.46 16.55 14.45 14.35 14.35 14.49 18.81 18.81 18.84 3.24 8.16 9.51 10.05 8.16 0.14 9.51 14.29 15.79	170-744 2012 2012 2012 2014 2015 2014 2006 2408 11.17 2017 2018 11.07 12.19 12.19 12.19 12.19 13.67 13.	
Group A B-Gal #1 B-Gal #2 B-Gal #2 B-Gal #4 AVG B18 #2 B18 #2 B18 #3 B18 #3 B18 #4 AVG SEM SEMY AS4-48 Group B B-Gal #1 B-Gal #2 B-Gal #2 B-Gal #3 B-Gal #4 B-Gal #2 B-Gal #3 B-Gal #4 AVG SEM STER #4 AVG STE	16.6 (Plates WGC 17.6 (16.0 (1	Hearts K: 17 10 16 21 17-5 12 33 35 39 25 33.0 29 Hearts K: 10 20 7 36 21.8 6,1	ANG #10*254 2.15 2.15 2.15 2.16 2.17 2.18 0.37 4.69 8.18 8.18 8.18 8.18 8.18 8.18 8.18 8.1	17.05. 17	12-244 12-24 10-45 11-29 12-29 12-29 12-29 12-29 12-29 12-29 12-39 12-49 13-46 14-46 1	5 1.9 5 1.0 1.0 1.0 1.0 1.0 1.0 1.0 1.0 1.0 1.0	11 10 4 7.3 1.9 15 15 15 15 15 15 15 15 15 15 15 15 15	#10-6A4 10.22 10.43 10.47 0.10 E.70 E.70 E.70 E.70 E.70 E.70 E.70 E.7	% 12.41 £.00 £.25 7.59 £.06 1.49 11.57 £.46 16.77 £.90 12.04 1.57 6.41 7.07 6.41 7.02 6.41 7.03 8.77	#10-64.d. UE7 0.85 0.87 0.87 0.15 1.04 0.15 1.04 0.05 0.70 0.05 0.77 0.77 0.77 0.77 0.77	46.6 48.8 48.4 48.4 48.4 48.4 48.4 53.2 53.2 52.8 52.8 52.8 53.8 53.8 53.8 53.8 53.8 53.8 53.8 53	TIONSMAL TIO	15.20 14.45 14.45 14.35 14.35 14.49 14.85 12.10 18.81 18.84 3.24 11.03 6.18 7.36 11.03 6.18 7.36 11.03 6.18 7.36 11.03 6.18 7.36 11.03 6.18 7.36 11.03 6.18 7.36 11.03 6.18	100-744 200-32 201-34 101-35 101-3	
Group A B-GM #1 B-GM #1 B-GM #2 B-GM #4 AVG B18 #2 B18 #2 B18 #2 B18 #3 AVG SEM AVG SEM AVG SEM AVG SEM AVG SEM B18 #2 B18 #3 B18 #4	110-3A4. 18.6 13.2 18.6 13.2 18.6 18.7 18.6 18.7 18.7 18.7 18.2 18.6 18.6 18.6 18.6 18.6 18.6 18.6 18.6	Hearts H	ANG # 0-24 2-15 2-15 2-16 2-17 3-26 8-18	Frenchs 50 77 77 77 77 77 57 57 57 56 59,0 7,0 60 57 74 68 69 57 71,2 65 59 59 57 71,2 65 59 59 59 59 59 59 59 59 59 59 59 59 59	#18-244. 12.53 - 10.43 14.50 12.39 12.79 0.48 7.95 10.00 8.27 8.15 0.43 ALC KIS *344 13.17 13.46 14.40 10.25 12.42 0.59 12.47 8.39 8.39	1.4 0.3 1.4 0.5 5 1 1 1 1 0.6 0.5 1 1 1 0.5 0.5 1 1 1 0.5 0.5 0.5 0.5 0.5 0.5 0.5 0.5 0.5 0.5	14 10 4 7.3 1.9 8 6.0 1.1 Monos % 8 7 7 3 8 8 .0 1.1 9 9 9 19 7 7	#10-644 10.22 10.45 10.47 0.10 E.70 E.70 E.70 E.70 E.70 E.70 E.70 E.7	% 12.41 8.00 8.20 7.89 8.06 1.49 11.47 18.77 8.80 12.04 1.67 7.07 8.87 7.07 8.41 7.62 6.41 7.62 6.41 7.62 6.41 7.63 8.41 8.57	#10-cent. 127 0.87 0.87 0.84 0.15 0.90 0.	44.44.44.44.44.44.44.44.44.44.44.44.44.	TIO-SALE TIO-SALE TIO-SALE TIOS TIOS TOS TOS TOS TOS TOS TOS TOS TOS TOS T	16.20 16.46 16.55 14.45 14.35 14.35 14.49 18.81 18.81 18.84 3.24 8.16 9.51 10.05 8.16 0.14 9.51 14.29 15.79	100-744 200-52 200-52 201-54 201-54 201-55 201-54 200-65 201-5	
Group A B-Gal #1 B-Gal #2 B-Gal #2 B-Gal #4 AVG B18 #2 B18 #2 B18 #2 B18 #3 B18 #4 AVG Croup B B-Gal #1 B-Gal #2 B-Gal #2 B-Gal #2 B-Gal #3 B-Gal #4 B-Gal #2 B-Gal #4 B-Gal #	168 (1986) 168 17.5 18.5 17.5 18.2 18.5 18.5 18.5 18.5 18.5 18.5 18.5 18.5	Neuts Neut	ANC MONTH AND	Lymphs 9: 90 70 71 72 72.5 50 82.0 50 82.0 7-06 82.0 7-06 82.0 7-06 82.0 7-06 82.0 7-06 82.0 82.0 82.0 82.0 82.0 82.0 82.0 82.0	12-244 12-24 10-45 11-29 12-29 12-29 12-29 12-29 12-29 12-29 12-39 12-49 13-46 14-46 1	5 1.9 5 1.0 1.0 1.0 1.0 1.0 1.0 1.0 1.0 1.0 1.0	14 10 4 7.3 1.9 6 7.3 8 6.0 1.1 8 8.0 1.1 9 9 19 9 19 9 19 9 19 9 11.0	#19-64-1 10-22 10-24 10-24 10-24 10-24 10-24 10-24 2-30 10-25 10-25 11-36 10-25 10-2	\$2.41 \$2.00 \$2.52 \$2.52 \$2.50 \$2.06 \$2.45 \$2.00 \$2.04 \$2.00 \$2.04 \$2.00	#10-64.4 U.F. O.S. O.S. O.S. O.S. O.S. O.S. O.S. O	445 445 444 444 445 445 445 445 445 445	TIONSMAL TIO	16.20 16.46 16.55 14.45 14.35 14.35 16.47 18.81 18.81 18.81 18.81 19.84 19.84 11.03 8.16 7.36 10.05 8.16 0.14 10.15 10.1	100-0444 20432 20432 20434 15136 16137 2044 2044 2044 2044 2044 1137 2445 1137 2445 1137 2445 1137 2445 1137 1450 150	
Group A B-GM #1 B-GM #1 B-GM #2 B-GM #4 AVG SEM B18 #2 B18 #2 B18 #2 B18 #3 Group B B-GM #1 AVG SEM AVG SEM AVG SEM B10 #1 B-GM #2 B-GM #4 AVG SEM SIN 8 #2 B-GM #4 SIN 8 #3 SIN 8 #4 SIN 9 #4 SI	VIBC MY 10-0AM 14.8 13.2 19.5 17.5 14.2 17.5 16.2 15.6 16.2 15.6 16.2 15.6 16.2 15.4 15.4 15.4 15.4 15.4 15.5 16.0 16.0 16.0 16.0 16.0 16.0 16.0 16.0	Hearts H	ANC MONTH AND	Cymphs 2:1 50 79 74 72 72.5 71 86 52.0 2.3 7-96 Lymphs 5:7 74 86 90 77.8 10.7	10 - 2 ALC 12.03 14.50 12.29 12.29 12.29 12.29 10.00 1	50 1.0 1.0 1.0 1.0 1.0 1.0 1.0 1.0 1.0 1.	14 10 4 7.3 1.8 8 6.0 1.1 Moreon % 7 3 8 6.0 1.1 9 9 11.0 2.7	## 10-24 10-25 10-25 10-25 10-45 10-47 10-47 10-47 10-47 10-47 10-56 10-	12.41 12.41 12.41 12.42 12.45 13	#10-644. US1 0.85 0.87 0.87 0.15 1.04 0.15 0.29 0.05 0.05 0.05 0.05 0.05 0.05 0.05 0.0	468 468 484 484 484 484 484 485 490 224 490 491 491 491 491 491 491 491 491 491 491	#1093/dc. 1409 1409 1308 1009 1100 11700 1100 11700 1100 1100 11	75 (15.20) 14.48 (16.55 14.23 15.40 15.40 15.40 15.40 15.40 15.40 15.40 15.40 15.40 15.40 15.50 15.73	100-24d 200-32 201-34 153.75 103.74 153.75 103.76 203.44 203.44 203.44 203.44 203.45 113.77 113.77 113.77 113.01 122.19 253.46 130.01 132.19 253.46 130.01 130.0	
Group A B-Gal #1 B-Gal #2 B-Gal #2 B-Gal #4 AVG B18 #2 B18 #2 B18 #2 B18 #3 B18 #4 AVG Croup B B-Gal #1 B-Gal #2 B-Gal #2 B-Gal #2 B-Gal #3 B-Gal #4 B-Gal #2 B-Gal #4 B-Gal #	VIBC MID AND AND AND AND AND AND AND AND AND AN	Neuts Neut	ANC HOST ANC AND	Lymphs 9: 90 70 71 72 72.5 50 82.0 50 82.0 7-06 82.0 7-06 82.0 7-06 82.0 7-06 82.0 7-06 82.0 82.0 82.0 82.0 82.0 82.0 82.0 82.0	10-24ct (12.83 - 14.50 (12.83 - 14.50 (12.83 - 14.60 (12.82 - 14.6	5 1.9 5 1.0 1.0 1.0 1.0 1.0 1.0 1.0 1.0 1.0 1.0	14 10 4 7.3 1.9 6 7.3 8 6.0 1.1 8 8.0 1.1 9 9 19 9 19 9 19 9 19 9 11.0	10-24 10-25 10-26 10-72 10-44 10-47 10-47 10-47 10-30	\$2.41 \$2.00 \$2.52 \$2.52 \$2.50 \$2.06 \$2.45 \$2.00 \$2.04 \$2.00 \$2.04 \$2.00	#10-64.d. U27 0.65 0.67 0.97 0.99 8.84 6.15 1.04 0.65 0.79 0.92 6.80 0.05 Abs Redics #10-64.d. 0.55 0.77 0.73 0.71 0.71 0.71 0.72 0.71 0.73 0.71 4.64 0.12 0.55 0.77 0.73 0.71 4.64 0.12 0.54 0.12 0.55 0.55 0.55 0.55 0.55 0.55 0.55 0.5	445 445 444 444 445 445 445 445 445 445	100344. 1400 1102 1122 1132 1008 1249 1700 11104 1104 1104 1104 1104 1106 1106 1	15.20 14.46 16.25 14.35 15.40 8.58 14.49 18.85 19.19 18.81 19.19 11.03 6.16 7.36 10.05 8.16 0.44 9.51 14.29 15.79 16.66 15.99 15.79 16.66 15.99 15.79 16.66 15.99 16.66 15.99	100-2044 20452 20452 20454 151,75 151,76 151,77 204,44 204,46 204,47 204,47 204,47 111,77 Abs RPR 111,77 Abs RPR 110,70 120,10 130,01 130,0	
Group A B-Gal 41 B-Gal 42 B-Gal 42 B-Gal 44 AVG SEM B18 42 B18 43 SEM AVG B-Gal 41 AVG B-Gal 42 B-Gal 44 AVG SEM SIB 81 B-Gal 44 AVG SEM SIB 81 B-Gal 44 AVG SEM SIB 81 SEM SEM SEM SEM SEM SEM SEM SE	VIBC MY 10-9AM 14.5 14.2 15.5 14.2 15.5 14.2 15.5 15.5 15.5 15.5 15.5 15.5 15.5 15	Hearts H	ANC HOME AND	Lymphs 7.5 60 82.7 77.5 75.7 66 82.0 22.3 7-66. Lymphs 7.6 63 9.7 61 63 9.7 61 63 9.7 61 63 9.7 61 64 64 65 65 65 65 65 65 65 65 65 65 65 65 65	10-24ct 12-81 14-50 12-81 14-50 12-9 12-9 12-9 12-9 12-9 12-9 12-9 12-9	600 % CO	11 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	110-64 10-22 10-42 10-42 10-42 10-42 10-42 4-74 10-42 10-42 11-36 10-52 10-52 9-78 10-52 10-52 9-78 10-52 10	12.41 12.41 12.41 12.41 12.42 13.45 11.47 12.42 12.44 13.47 13	#10-64-44 US7 0.87 0.87 0.15 1.046 0.15 0.20 0.05 0	468 468 484 484 484 484 485 400 224 220 224 20 224 40 40 40 40 40 40 40 40 40 40 40 40 40	#10*3/46. #100** #100*	16.20 14.48 16.58 14.33 15.40 6.58 14.43 15.10 18.81 18.81 18.81 18.10 18.11 18.11 18.11 19.11 1	100-244 20432 20432 20144 15325 2044 2044 2044 2044 2044 2044 1117 1117 1500 11219 2530 11219 2530 1134 1134 1134 1134 1134 1134 1134 11	
Group A B-Gal #1 B-Gal #2 B-Gal #2 B-Gal #4 AVG B18 #2 B18 #2 B18 #2 B18 #2 B18 #3 B16 #4 AVG B-Gal #1 B-Gal #1 B-Gal #1 B-Gal #2 B-Gal #4	VIBC MID NO NEW MID NO NEW MID	Hearts H	ANC HOME AND ANC AND	Lymphs 9. 19 70 71 72 72.5 77 75 76 82.0 2.1 706 Lymphs 76 60 60 60 77.8 61 63 63 64 65 67 67	10-24d. 12.83 14.50 12.79 12.79 12.79 12.79 12.79 12.79 12.70 12.71 12.71 12.71 12.72 12.72 12.72 12.72 12.73 12.77 12.7	5 1.9 1.9 1.9 1.9 1.9 1.9 1.9 1.9 1.9 1.9	11 4 10 4 7.3 1.9 5 7 3 8 6.0 1.1 1.1 9 9 9 9 9 9 7 11.0 2.7 Monos % 10	10-24 10-25 10-26 10-27 10-44 10-47 10-47 10-47 10-47 10-47 10-57 10-58	12.41 12.41 12.41 12.42 12.57 12.95	#10-64.d. 1.27 0.65 0.67 0.79 0.09 0.00 0.00 0.00 0.00 0.00 0.0	46.8 46.8 46.8 46.8 46.8 46.8 46.8 46.8	**T0*344.** **T0*344.** 1460.** 1460.** 1360.** 1376.*	15.20 14.46 16.25 14.35 15.40 8.58 14.49 18.85 19.19 18.81 19.19 11.03 6.16 7.36 10.05 8.16 10.05 10.05 14.49 15.73 16.56 15.73 16.56 15.73 16.56 16.5	#10 - 24/44, 204.32 224.34 153.25 163.27 224.36 153.27 240.36 113.77 240.36 240	
Group A B-Gal #1 B-Gal #2 B-Gal #2 B-Gal #4 AVG SEM B18 #2 B18 #2 B18 #3 AVG SEM AVG SEM AVG SEM AVG SEM AVG SEM AVG SEM B-Gal #1 AVG B-Gal #1 AVG B-Gal #1 AVG SEM STAN AVG B-Gal #1	VIBC VIDCA V	Hearts H	ANC 1102 2.11 2.14 2.15 2.11 2.14 2.15 2.11 2.14 2.15 2.17 2.15 2.18 2.18 2.18 2.18 2.18 2.18 2.18 2.18	Lymphs 75 80 80 77 77 77 75 66 80 80 80 76 80 80 77 77 76 80 80 77 77 81 80 80 80 80 80 80 80 80 80 80 80 80 80	## 19-264 10-43 14-50 12-31 14-50 12-32 12-32 10-33 10-33 13-17	2.0 1.3 1.3 1.3 1.3 1.3 1.3 1.3 1.3 1.3 1.3	11 4 14 14 15 15 15 15 15 15 15 15 15 15 15 15 15	110-644 10-42 10-42 10-43 10-44 10-47 10-47 10-47 10-47 10-48 10-4	12.41 12.41 12.41 12.41 12.42 13.52 13	#10-64-4. US7 0.87 0.87 0.87 0.15 1.046 0.15 1.046 0.79 0.22 0.05 0.05 0.70 0.05 1.01 0.05 0.77 0.77 0.77 0.77 0.77 0.77 0.77	# 46.6 46.6 46.6 46.6 68.6 60.6	#1009/Mc. 1400 1410 1300 1300 1240 11700 11700 11700 1410 1410 1410 1410	16.20 14.48 16.58 14.35 15.40 6.58 14.43 29.10 18.81 18.81 18.81 18.13 6.16 7.36 6.16 7.36 11.03 8.16 0.44 9.51 14.29 15.79 16.66 14.96 16.96 16.96 16.96 16.96 16.96 16.96 16.96 16.96 16.96 16.96 16.96 16.96 16	100-2444 20432 20432 20144 15325 20444 20446	
Group A B-Gal #1 B-Gal #1 B-Gal #2 B-Gal #3 B-Gal #4 AVG B18 #2 B18 #3 B18 #4 AVG B-Gal #1 B-Gal #1 B-Gal #2 B-Gal #3 B-Gal #4 B-Gal #1 B-Gal #1 B-Gal #1 B-Gal #1 B-Gal #1 B-Gal #1	VIBO M. 10-30 M. 10-3	Neutra N	ANC HOME STATE STA	Lymphs 9. 93 79 79 74 72 72.5 55 57 66 89.0 2.1 60 60 67 71.2 7.0 61 62 63 63 64 65 65 67 67 69 62 62 67 68 62 67 68 68 68 68 68 68 68 68 68 68 68 68 68	110-24-1 112-83 114-50 112-9 114-50 112-9	19 19 19 19 19 19 19 19 19 19 19 19 19 1	11 4 4 7.5 1.9 7 3 8 6.0 1.1 Monos % 8 7 9 9 19 7 11.0 2.7 Monos % 11.1 9 9 19 7 11.0 2.7 Monos % 12 12 4	#19-644 10-22 10-40 10-72 10-44 10-47 10-47 10-47 10-64 ####################################	12.41 12.41 12.41 12.42 12.57 12.57 12.42 11.57 12.04 1.57 1.57 1.57 1.57 1.57 1.57 1.57 1.57	#10-64.d. #10-65. #10-65. #10-65. #10-65. #10-65. #10-65. #10-65. #10-66. #	# 468 468 468 468 668 668 668 668 668 668	**T0*344.** **T0*344.** 1460.** 1460.** 1470.** 1370.** 1370.** 1370.** 1410.*	15.20 14.46 16.25 14.35 15.40 18.85 12.19 18.81 12.44 3.24 3.24 3.24 3.24 3.24 3.24 3.24	#10 - 24/44, #15.25 224,54 15.25 224,54 15.25 224,55 224,55 24,55	
Group A B-Gal #1 B-Gal #2 B-Gal #2 B-Gal #4 AVG SEM B18 #2 B18 #2 B18 #3 AVG SEM AVG B18 #1 B-Gal #1 AVG B18 #1 B-Gal #1	VIBC VIDCA V	Hearts H	ANC 1102 2.11 2.14 2.15 2.11 2.14 2.15 2.11 2.14 2.15 2.17 2.15 2.18 2.18 2.18 2.18 2.18 2.18 2.18 2.18	Lymphs 7.2 7.2 7.5 66 83.0 7.7 7.5 66 83.0 7.6 83.0 7.6 83.0 7.6 83.0 83.0 83.0 10.7 14.66 Lymphs 67 68 83.0 10.7 14.66 Lymphs 67 85 85 85	10-24ct 12-25: 14-50 12-25: 10-25: 12	14 03 14 05 15 15 15 15 15 15 15 15 15 15 15 15 15	11 4 4 10 4 7.5 1.9 5 6.0 1.1 1.1 1.2 1.2 1.2 1.2 1.2 1.2 1.2 1.2	### ### ### ### ### #### #### ########	12.41 12.41 12.41 12.42 12.52 12.52 12.62 12.64	#10-64.4. US7 0.87 0.87 0.87 0.89 0.15 1.046 0.15 1.046 0.05 0.79 0.05 4.00 0.05 Abs Redcs #10-64.1 0.15 0.17 0.17 0.17 0.17 0.17 0.17 0.17 0.17	#4. 46.4 46.4 46.4 46.4 46.4 46.4 46.4 4	#1005/Me. 1400 14100 1300 1300 1240 1100 891 1100 894 1418 1254 1418 1250 1418 1250 1618 1250 1618 1250 1618 1707 1707 1708 1709 1709 1709 1709 1709 1709 1709 1709	15.20 14.48 14.53 14.53 15.40 8.58 14.83 29.10 18.83 29.10 18.83 7.30 8.18 7.30 8.18 10.05 8.18 10.05 8.18 14.29 15.70 14.29 15.70 14.50 1	100-2444 206.32 206.32 206.32 206.34 207.32 207.	
Group A B-Gal #1 B-Gal #1 B-Gal #2 B-Gal #3 B-Gal #4 AVG B18 #2 B18 #3 B18 #4 AVG B-Gal #1 B-Gal #1 B-Gal #2 B-Gal #3 B-Gal #4 B-Gal #1 B-Gal #1 B-Gal #1 B-Gal #1 B-Gal #1 B-Gal #1	10-3A4 14.0 15.4 11.5 11.5 11.5 11.5 11.5 11.5 11.5	Neutra N	ANC HOME STATE STA	Lymphs 9. 93 79 79 74 72 72.5 55 57 66 89.0 2.1 60 60 67 71.2 7.0 61 62 63 63 64 65 65 67 67 69 62 62 67 68 62 68 68 68 68 68 68 68 68 68 68 68 68 68	110-24-1 112-83 114-50 112-9 114-50 112-9	19 19 19 19 19 19 19 19 19 19 19 19 19 1	11 4 4 7.5 1.9 7 3 8 6.0 1.1 Monos % 8 7 9 9 19 7 11.0 2.7 Monos % 11.1 9 9 19 7 11.0 2.7 Monos % 12 12 4	#19-644 10-22 10-40 10-72 10-44 10-47 10-47 10-47 10-64 ####################################	12.41 12.41 12.41 12.42 12.57 12.57 12.42 11.57 12.04 1.57 1.57 1.57 1.57 1.57 1.57 1.57 1.57	#10-64.4. UE7 0.65 0.67 0.79 0.84 0.15 1.04 0.65 0.79 0.92 0.80 0.80 0.77 0.73 0.77 0.77 0.77 0.77 0.77 0.7	#6. 44.8 44.4 44.4 45.4 4.7 4.7 4.7 4.7 4.7 4.7 4.7 4.7 4.7 4	**T0*344.** **T0*344.** **1463.** **1463.** **1378.** **1378.** **1378.** **1378.** **1378.** **1378.** **1378.** **1378.** **1378.** **1283.** **	15.20 14.46 16.25 14.35 15.40 8.58 14.49 18.85 19.19 18.81 19.24 3.24 3.24 3.24 3.24 3.24 3.24 3.24 3	#10 **M. 120.22 120.32 120.32 120.32 120.32 120.32 120.32 120.32 120.32 120.32 130.32 1	
Group A B-Gal #1 B-Gal #1 B-Gal #2 B-Gal #4 AVG B18 #1 B18 #2 B18 #3 B18 #4 AVG B18 #3 B-Gal #1 B-Gal #1 B-Gal #1 B-Gal #1 B-Gal #2 B-Gal #1 B-Gal #2 B-Gal #1 B-Gal #2 B-Gal #2 B-Gal #2 B-Gal #1 B-Gal #2 B-Gal #1 B-Gal #1 B-Gal #2 B-Gal #1	VIBO M. 10-30 M. 10-3	Hearts H	ANC 110 2.16 2.16 2.16 2.16 2.16 2.16 2.16 2.16	Lymphs 7.2 72.5 77.7 72.5 77.7 75.5 77.6 82.0 1.7.06 1.1.7 7.1.8 83.0 83.0 83.0 83.0 83.0 83.0 83.0 83	10-24ct 12-251 1	23 14 25 15 15 15 15 15 15 15 15 15 15 15 15 15	11 4 4 10 4 7.5 1.9 5 6.0 1.1 1.1 1.2 1.2 1.2 1.2 1.2 1.2 1.2 1.2	### ### ### ##########################	12.41 12.41 12.41 12.42 12.42 13.75 14.95 14.91 15.77 12.04 15.77 12.04 15.77 12.04 15.77 12.04 15.77 10.33 10.34 10.35 10	#10-644. ADS GAS GA	#4.44.45.45.45.45.45.45.45.45.45.45.45.45	#10-3/44. **1460 **1470 **1370 **1370 **1064 **1070 **107	15.20 14.48 14.53 14.53 15.40 8.58 14.83 29.10 18.83 29.10 18.83 8.18 7.30 8.18 7.30 8.18 10.05 14.29 15.73 14.29 15.73 14.29 15.73 14.29 15.73 14.29 15.73 14.29 15.73 14.29 15.73 14.29 15.73 16.69 14.59	100-2444 206.32 206.32 206.32 206.34 207.32 207.	
Group A B-Gal #1 B-Gal #1 B-Gal #2 B-Gal #3 B-Gal #4 AVG B18 #3 B-Gal #1 B-Gal #1 B-Gal #1 B-Gal #2 B-Gal #2 B-Gal #3 B-Gal #4 AVG B18 #3 SNOY AS4-#8 B-Gal #1 B-Gal #1 B-Gal #3 B-Gal #4 AVG B-Gal #3 B-Gal #3 B-Gal #4 B-Gal #4 AVG B-Gal #3 B-Gal #4 B-Gal #4 AVG B-Gal #4	VIBC VIDCA V	Hearts H	ANC 102 103 103 104 105	Lymphs 9. 69 77 77 78.5 65 89.0 23 7-06. Lymphs 74 66 69 77 71.5 67 68 89 81 10.7 14-66. Lymphs 67 67 68 89 89 81 81 81 81 81 81 81 81 81 81 81 81 81	ALC 12.52 12	23 14 25 15 15 15 15 15 15 15 15 15 15 15 15 15	11 4 4 10 4 7.5 1.9 5 6.0 1.1 1.1 1.2 1.2 1.2 1.2 1.2 1.2 1.2 1.2	### ### ##############################	12.41 12.41 12.41 12.42 12.42 12.42 11.97 12.04 1.57 12.04 1.57 12.04 1.57 12.04 1.57 12.04 1.57 12.04 1.57 12.04 1.57 12.04 1.57 1.5	#10-644. QUS	#6.48.44.44.44.45.45.45.45.45.45.45.45.45.45.	**T0*344.** **T0*344.** **1463.** **1463.** **1378.** **1378.** **1378.** **1378.** **1378.** **1378.** **1378.** **1378.** **1378.** **1378.** **1282.** **	15.20 14.46 16.25 14.35 15.40 8.58 14.49 18.85 19.19 18.81 19.19 11.03 6.16 7.36 10.05 10.	#10 - 24/44, #15.25 224,34 15.25 19.37 25.26 22 206.44 200.96 206.00 11.37 240.85 11.37 240.85 11.37 25.29 25.20 26.24 200.96 206.00 11.37 15.24 15.26 20.26 206.00 11.37 15.24 15.26 20.26 217.27 181.20 217.27 217	
Group A B-Gal #1 B-Gal #1 B-Gal #2 B-Jan #3 B-Jan #4 B-Jan #	#186 M	Neurita Neur	ANC HOSPAN ANC AND	Lymphs 7.2 72.5 77.7 72.5 77.7 75.5 77.6 82.0 1.7.06. 1.4.06 1.4.07 1.4.06 87.6 82.6 87.6 82.6 87.6 82.6 87.6 82.6 83.6 84.6 85.6 87.6 87.6 87.6 87.6 87.6 87.6 87.6 87	## 12-254 ## 12-25 ##	20 2 1.3 1.5 1.5 1.5 1.5 1.5 1.5 1.5 1.5 1.5 1.5	Monos % 8.0 1.1 9 9 19 7 11.0 2.7 Monos % 12 4 4 7.5 2.1 7 6 5	### ### ##############################	Retter 123	#10-64.4. UE7 0.65 0.67 0.97 0.99 8.44 0.15 1.04 0.05 0.79 0.92 0.80 0.05 0.77 0.73 0.71 0.71 0.72 0.73 0.71 0.75 0.75 0.77 0.73 0.71 0.75 0.75 0.77 0.73 0.71 0.75 0.75 0.77 0.73 0.71 0.75 0.75 0.77 0.73 0.71 0.75 0.75 0.75 0.75 0.75 0.75 0.75 0.75	#4.44.45.45.45.45.45.45.45.45.45.45.45.45	#10-3/44. **1460 **1470 **1370 **1370 **1064 **1070 **107	15.20 14.48 14.53 14.53 15.40 8.58 14.83 29.10 18.83 29.10 18.83 8.18 7.30 8.18 7.30 8.18 10.05 14.29 15.73 14.29 15.73 14.29 15.73 14.29 15.73 14.29 15.73 14.29 15.73 14.29 15.73 14.29 15.73 16.69 14.59	#10-9444 #10-9444 #10-12-20-12-20-12-20-14-20-20-20-20-20-20-20-20-20-20-20-20-20-	
Group A B-Gal #1 B-Gal #1 B-Gal #2 B-Gal #4 AVG SEM AVG B-Gal #1 AVG SEM AVG B-Gal #1 AVG SEM AVG B-Gal #1 AVG B-Gal #4 AVG SEM AVG B-Gal #4 AVG B-Gal #4 AVG B-Gal #4 AVG SEM AVG SEM B-Gal #4 AVG SEM B-Gal #4 AVG SEM B-Gal #1 AVG SEM B-Gal #4 AVG SEM B-Gal #4 AVG B-Gal #4 B-	10-3A4 14.0 15.4 15.4 15.4 15.4 15.4 15.4 15.4 15.4	Hearts H	ANC 110 2.16 2.16 2.16 2.16 2.16 2.16 2.17 2.16 2.17 2.16 2.17 2.16 2.17 2.17 2.17 2.17 2.17 2.17 2.17 2.17	Lymphs 7.2 7.2 7.5 60 80.0 7.7 7.5 7.5 66 80.0 2.3 7.66 Lymphs 7.6 60 80.0 2.3 7.6 61 62 63 61 62 63 61 65 65 65 65 65 65 65 65 65 65 65 65 65	## 12-251 ## 12-251	5 - 1 - 2 - 2 - 2 - 2 - 2 - 2 - 2 - 2 - 2	Monos 11 4 7.3 1.9 6.0 6.0 1.1 Monos 1.1 2.7 Monos 1.2 4 7.3 5 6 6 6 6 6 6 7 7 8 8 8 8 8 8 8 8 8 8 8	### ### ### ### ### ### ### ### ### ##	Reflex K. 1.23 Reflex K. 1.25 Reflex	#10-64.4 US1 OLS2 OLS2 OLS3 OLS3 OLS3 OLS4 OLS5 OLS6 OLS9 OLS6 OLS9 OLS6 OLS9 OLS6 OLS7 OLS7 OLS7 OLS7 OLS7 OLS7 OLS7 OLS7	#4.84 48.4 48.4 48.4 48.4 48.4 48.4 48.4	#10-3/44. #1460 #1470 #1470 #1570 #	15.20 14.48 14.53 14.53 14.53 15.40 15.40 16.83 29.10 18.81 18.81 18.81 19.41 11.02 11.02 15.73 14.29 15.73 14.29 15.73 14.29 15.73 14.29 15.73 14.29 15.73 14.29 15.73 14.29 15.73 14.29 15.73 16.66 14.59 15.73 16.66	100-744 120-52 120-52 120-54 130-56 130-7	
Group A B-Gal #1 B-Gal #1 B-Gal #2 B-Gal #4 AVG B18 #2 B18 #3 B18 #3 B18 #3 B18 #4 AVG B-Gal #1 B-Gal #2 B-Gal #3 B-Gal #4 AVG B18 #3 B18 #4 B-Gal #3 B-Gal #4 AVG B-Gal	#186 M 196 M	Neurita Neur	ANC 1102 2.16 3.16 3.16 3.16 3.16 3.17 4.07	Lymphs 72 72 72 73 75 77 77 75 77 75 77 85 82.0 82.0 77 71.2 77 85 87 87 87 87 87 87 87 87 87 87 87 87 87	## 12-25 ## 12-25	23 14 25 15 15 15 15 15 15 15 15 15 15 15 15 15	Monos % 8.0 1.1 9 9 19 7 11.0 2.7 Monos % 12 4 4 7.5 2.1 7 6 6.0 6.0 6.0 6.0 6.0 6.0 6.0 6.0 6.0 6	### ### ##############################	Retics 4.15 1.20 1.20 1.20 1.20 1.20 1.20 1.20 1.20	### RedGe 1.12 CAS	# 448 448 448 448 448 448 448 448 448 44	#10*344. #10*344. #1463. #1463. #1463. #1463. #1463. #1464. #1	15.20 14.46 14.25 14.45 14.45 14.45 15.40 6.58 14.45 18.85 19.10 18.81 19.44 3.24 3.24 3.24 3.24 3.24 3.24 3.24 3	#10-944 20452 185.32 271.44 185.25 286.44 200.66 286.03 286.44 200.66 286.03 11.17 266.03 12.19 12.19 12.19 12.19 13.66 13.6	
Group A B-Gal #1 B-Gal #1 B-Gal #2 B-Gal #4 AVG SEM AVG B-Gal #1 AVG SEM AVG B-Gal #1 AVG SEM AVG B-Gal #1 AVG B-Gal #4 AVG SEM AVG B-Gal #4 AVG B-Gal #4 AVG B-Gal #4 AVG SEM AVG SEM B-Gal #4 AVG SEM B-Gal #4 AVG SEM B-Gal #1 AVG SEM B-Gal #4 AVG SEM B-Gal #4 AVG B-Gal #4 B-	10-3A4 14.0 15.4 15.4 15.4 15.4 15.4 15.4 15.4 15.4	Hearts H	ANC 110 2.16 2.16 2.16 2.16 2.16 2.16 2.17 2.16 2.17 2.16 2.17 2.16 2.17 2.17 2.17 2.17 2.17 2.17 2.17 2.17	Lymphs 7.2 7.2 7.5 60 80.0 7.7 7.5 7.5 66 80.0 2.3 7.66 Lymphs 7.6 60 80.0 2.3 7.6 61 62 63 61 62 63 61 65 65 65 65 65 65 65 65 65 65 65 65 65	## 12-251 ## 12-251	5 - 1 - 2 - 2 - 2 - 2 - 2 - 2 - 2 - 2 - 2	Monos 11 4 7.3 1.9 6.0 6.0 1.1 Monos 1.1 2.7 Monos 1.2 4 7.3 5 6 6 6 6 6 6 7 7 8 8 8 8 8 8 8 8 8 8 8	### ### ### ### ### ### ### ### ### ##	Reflex K. 1.23 Reflex K. 1.25 Reflex	#10-64.4 US1 OLS2 OLS2 OLS3 OLS3 OLS3 OLS4 OLS5 OLS6 OLS9 OLS6 OLS9 OLS6 OLS9 OLS6 OLS7 OLS7 OLS7 OLS7 OLS7 OLS7 OLS7 OLS7	#4. #4. #4. #4. #4. #4. #4. #4. #4. #4.	**T0**344.** **T0**344.** **1400 **1300 **1020 **1020 **104 **104 **294 **1418 **1294 **1418 **1294 **1418 **1294 **1418 **1294 **1418 **1294 **1418 **1294 **1418 **1294 **1418 **1294 **1418 **1294 **1418 **1294 **1418 **1294	15.20 14.48 16.58 14.35 14.35 15.40 6.58 18.10 7.36 11.03 8.10 7.36 10.05 11.03 8.10 10.05 11.03 8.10 10.05 11.03 8.11 10.05 11.03 8.14 9.44 11.03 10.05 11.03 10.03 10.	#10-2444 120-32 121-34 131-35 131-	

Table II: Bone marrow and Splenic Hematopoietic Progenitors

5

10

15

20

25

	CFU	-MEG	BFU-E			
Bone Marrow*	B-Gal	B18	B-Gal	B18	B-Gal	B18
Day 7	16.0 ± 3.5	15.7 ± 3.1	307 ±117	241±78	51 ± 19	25 ± 11
Day 14	10.7 ± 2.3	15.3 ± 1.2	233 ± 15	373±35	30 ± 10	60 ± 30
Day 21	5.7 ± 0.6	6.7 ± 3.1	170 ± 17	160±27	40 ± 10	27 ± 6
Spleen**						
Day 7	9.3 ± 1.6	19.5 ± 1.5	27 ± 3	298 ± 6	1.3 ± 1.2	68 ± 10
Day 14	9.7 ± 0.6	12.7 ± 0.6	267 ± 32	197 ±21	33 ± 6	10 ± 10
Day 21	17.0 ± 1.0	19.3 ± 2.5	187±6	73 ± 15	23 ± 6	23 ± 6

Hematopoietic precursors were determined form pooled spleen and bone marrow samples from four animals in each group. For quantitation of CFU-GM and BFU-E, either 1 x 10⁴ bone marrow cells or 1 x 10⁵ spleen cells were added to complete alpha methylcelluose medium (0.9% methylcellulose in alpha medium, 30% fetal bovine serum, 1% bovine serum albumin, 10-4M 2-mercaptoethanol, 2 mM L-glutamine, 2% murine spleen cell conditioned medium, and 3 U/mL erythropoietin) and aliquoted into 35 mm tissue culture dishes in a final volume of 1.0 mL. Cultures were incubated for 7 days at 37°C, and 5% CO₂. Microscopic colonies were defined as clusters of 50 or more cells. For quantitaion of CFU-MEG, either 1 x 10⁵ bone marrow cells or 1 x 10⁶ spleen cells were added to complete alpha methylcellulose medium and incubated as described above. Megakaryocyte colonies were defined as a group of 3 or more cells.

^{*}Bone marrow progenitors are represented as mean ± sd number of colonies per 10⁵ cells.

^{**}Spleen progenitors are represented as mean ± sd number of colonies per 10⁶ cells.

Table III: Spleen Weights and Femur Cellularity

Spleen Wt. (Mg)	B-Gal	B18		Femur Cellularity (x10 ⁶)	B-Gal	B18
Day 7	187 ± 19	224 ± 29	1	Day 7	28	23
Day 14	175 ± 13	170 ± 10		Day 14	28	27
Day 21	174 ± 21	151 ± 27		Day 21	28	26

Spleen weights were determined at time of sacrifice are represented as means \pm sd from four animals.

Table IV: Bone Marrow Myeloid: Erythoid Ratios

5

10

15

20

25

30

Group	Mouse #	Day 7	Day 14	Day 21	
B-gal	1	1.43	2.12	5.78	
	2	0.91	2.46	5.83	
	3	1.62	1.03	3.66	
	4		5.44	4.82	
AVG		1.32	2.76	5.02 1.89	
SD		0.37	0.37		
B-gal	1	5.59	2.01	2.02	
	2	6.51	1.25	2.13	
	3	5.49	1.58	1.81	
	4	0.50	2.51	2.92	
AVG		4.52	1.86	2.22	
SD		1.29	2.72	0.56	

All entries represent the number of myeloid cells per 1 erythroid cell. Normal mouse ratios are approximately 1:1 to 2:1.

Example 5

Additional Experiments Relating to

Hematopoietic Activity of Human CTLA-8

B18 (human CTLA-8) was tested for the ability to induce production of factors having hematopoietic activity in a factor-dependent cell proliferation assay using the

human erythroleukemic cell line, TF-1 (Kitamura et al., J. Cell Physiol. 140:323 (1989)). The cells were initially grown in the presence of rhGMCSF (100 U/ml). The cells were fed three days prior to setting up the assay. The assay conditions were as follows:

5	cells/well	5000/200µ1
	incubation time	3 days
	pulse time	4 hours
	amount of tritiated thymidine	0.5µCi/well
	counting time	1 minute-
10	replicates	2

B18 alone, conditioned medium (CM) from B18 induced HS-5 cells were assayed. Buffer alone, CM from HS-5 cells induced with buffer and CM from uninduced HS-5 cells were assayed as controls. Results are shown in Fig. 5. B18 (human CTLA-8) demonstrated an abilit to induce production of factors which induced TF-1 proliferation. This activity was substantially eliminated by the addition of anti-GMCSF antibodies. These data demonstrate that human CTLA-8 (B18) is able to induce hematopoiesis. Particularly, without being bound by any theory, it appears that human CTLA-8 (B18) induces production of GM-CSF and/or IL-3.

20 Example 6

15

25

Ability of Human CTLA-8 to Induce Production of IL-6 and IL-8

MRC5 cells were incubated in the presence of human CTLA-8 (B18) and production of IL-6 and IL-8 were measured. Herpes CTLA-8 (IL-17) was used as a positive control. Applicants' human CTLA-8 (B18) demonstrated titratable production of both IL-6 and IL-8 (see Figs. 6 and 7).

All patent and literature references cited herein are incorporated by reference as if fully set forth.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

- (i) APPLICANT: Jacobs, Kenneth Kelleher, Kerry Carlin, McKeough Goldman, Samuel Pittman, Debra Mi, Sha Neben, Steven Giannotti, JoAnn Golden'Fleet, Margaret
- (ii) TITLE OF INVENTION: Human CTLA-8 and Uses of CTLA-8-Related Proteins
 - (iii) NUMBER OF SEQUENCES: 9
 - (iv) CORRESPONDENCE ADDRESS:
 - (A) ADDRESSEE: Genetics Institute, Inc.(B) STREET: 87 CambridgePark Drive

 - (C) CITY: Cambridge
 - (D) STATE: Massachusetts
 - (E) COUNTRY: USA
 - (F) ZIP: 02140
 - (v) COMPUTER READABLE FORM:
 - (A) MEDIUM TYPE: Floppy disk

 - (B) COMPUTER: IBM PC compatible (C) OPERATING SYSTEM: PC-DOS/MS-DOS
 - (D) SOFTWARE: PatentIn Release #1.0, Version #1.25
 - (vi) CURRENT APPLICATION DATA:
 - (A) APPLICATION NUMBER:
 - (B) FILING DATE:
 - (C) CLASSIFICATION:
 - (viii) ATTORNEY/AGENT INFORMATION:

 - (A) NAME: Brown, Scott A.
 (B) REGISTRATION NUMBER: 32,724
 - (C) REFERENCE/DOCKET NUMBER: G15262
 - (ix) TELECOMMUNICATION INFORMATION:
 - (A) TELEPHONE: (617) 498-8224
 - (B) TELEFAX: (617) 876-5851
- (2) INFORMATION FOR SEQ ID NO:1:
 - (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 813 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: double
 - (D) TOPOLOGY: linear
 - (ii) MOLECULE TYPE: cDNA
 - (iii) HYPOTHETICAL: NO
 - (ix) FEATURE:

 - (A) NAME/KEY: CDS
 (B) LOCATION: 56..544

	(xi)	SE	QUEN	CE DI	ESCR!	PTIC	on: s	SEQ :	ID NO	0:1:		٠				
GGG <i>I</i>	AGA1	rac i	ATTC	ACAG	AA AG	GAGC	PTCC'	r gc	ACAA	AGTA	AGC	CACCI	AGC (GCAAG	ATG Met 1	58
ACA Thr	GTG Val	AAG Lys	ACC Thr 5	CTG Leu	CAT His	GGC Gly	CCA Pro	GCC Ala 10	ATG Met	GTC Val	AAG Lys	TAC Tyr	TTG Leu 15	CTG Leu	CTG Leu	106
TCG Ser	ATA Ile	TTG Leu 20	GGG Gly	CTT Leu	GCC Ala	TTT Phe	CTG Leu 25	AGT Ser	GAG Glu	GCG Ala	GCA Ala	GCT Ala 30	CGG Arg	AAA Lys	ATC Ile	154
CCC Pro	AAA Lys 35	GTA Val	GGA Gly	CAT His	ACT Thr	TTT Phe 40	TTC Phe	CAA Gln	AAG Lys	CCT Pro	GAG Glu 45	AGT Ser	TGC Cys	CCG Pro	CCT Pro	202
GTG Val 50	CCA Pro	GGA Gly	GGT Gly	AGT Ser	ATG Met 55	AAG Lys	CTT Leu	GAC Asp	ATT Ile	GGC Gly 60	ATC Ile	ÀTC Ile	AAT Asn	GAA Glu	AAC Asn 65	Ź50
CAG G1n	CGC Arg	GTT Val	TCC Ser	ATG Met 70	TCA Ser	CGT Arg	AAC Asn	ATC Ile	GAG Glu 75	AGC Ser	CGC Arg	TCC Ser	ACC Thr	TCC Ser 80	CCC Pro	298
TGG Trp	AAT Asn	TAC Tyr	ACT Thr 85	GTC Val	ACT Thr	TGG Trp	GAC Asp	CCC Pro 90	AAC Asn	CGG Arg	TAC Tyr	CCC Pro	TCG Ser 95	GAA Glu	GTT Val	346
GTA Val	CAG Gln	GCC Ala 100	CAG Gln	TGT Cys	AGG Arg	Asn	TTG Leu 105	GGC Gly	TGC Cys	ATC Ile	AAT Asn	GCT Ala 110	CAA Gln	GGA Gly	AAG Lys	394
GAA Glu	GAC Asp 115	ATC Ile	TCC Ser	ATG Met	AAT Asn	TCC Ser 120	GTT Val	CCC Pro	ATC Ile	CAG Gln	CAA Gln 125	GAG Glu	ACC Thr	CTG Leu	GTC Val	442
														GAG Glu		490
														CAC His 160		538
GTG Val		TAAG	GAGG1	rgc A	TATO	CACT	C AC	ectg <i>i</i>	\AGA/	A GCT	'GTAC	JAAA	TGC	CACTO	CT	594
TACC	CAGI	GC 1	CTG	CAACA	AA G1	CCTC	TCTC	ACC	CCCZ	\ATT	CCCI	CCAC	TT (CACAC	GACTC	654
LATT)AAT	SAC C	CTGC	ACGG1	AT GO) A A A E	CAGA	AA A	YTTAT	CACA	ATG	ratgi	rgr (GTATO	STACTA	714
CACI	'TTA'	TAT T	rtgat	PATCT	TA AZ	\ATG1	TAGO	AG?	\AAA!	ATTA	ATAT	PATTO	CAG '	TGCT	\ATATA	774
ATA	lagt?	ATT A	LATA!	\TTT?	LA AA	LATA	LAAA /	AA A	LAAA	AAA						813

(2) INFORMATION FOR SEQ ID NO:2:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 163 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: protein

Wot								: SE				• • • • •		•	_	
1	THE	Val	Lys	5	reu	піѕ	GIY	Pro	10	Met	vai	гĀЗ	туr	15	Leu	
Leu	Ser	Ile	Leu 20	Gly	Leu	Ala	Phe	Leu 25	Ser	Glu	Ala	Ala	Ala 30	Arg	Lys	
Ile	Pro	Lys 35	Val	Gly	His	Thr	Phe 40	Phe	Gln	Lys	Pro	Glu 45	Ser	Суѕ	Pro	
Pro	Val 50	Pro	Gly	Gly	Ser	Met 55	Lys	Leu	Asp	Ile	Gly 60	Ile	Ile	Asn	Glu	
Asn 65	Gln	Arg	Val	Ser	Met 70	Ser	Arg	Asn	Ile	Glu 75	Ser	Arg	Ser	Thr	Ser 80	
Pro	Trp	Asn	Tyr	Thr 85	Val	Thr	Trp	Asp	Pro 90	Asn	Arg	Tyr	Pro	Ser 95	Glu	
Va1	Val	Gln	Ala 100	Gln	Суз	Arg	Asn	Leu 105	Gly	Суѕ	Ile	Asn	Ala 110	Gln	Gly	
Lys	Glu	Asp 115	Ile	Ser	Met	Asn	Ser 120	Val	Pro	Ile	Gln	Gln 125	Glu	Thr	Leu	
Val	Val 130	Arg	Arg	Lys	His	Gln 135	Gly	Cys	Ser	Val	Ser 140	Phe	Gln	Leu	Glu	
Lys 145	Val	Leu	Val	Thr	Val 150	Gly	Cys	Thr	Cys	Val 155	Thr	Pro	Val	Ile	His 160	
His	Val	Gln														
(2)	INFO	DRMA!	PION	FOR	SEQ	ID N	10:3	:								
	(i)	() (I ()	QUENC A) Li 3) T' C) S'	INGTI (PE : [RANI	H: 46 nucl	il ba leic ESS:	acio doul	pairs i	5							
	(ii)	•	LECUI													
1	(iii)	HYI	отні	etic:	AL: 1	10										
	(ix)	(2	ATURI A) NA B) LO	ME/I			155									
	(xi)) SE(QUENC	E DI	ESCRI	PTIC	ON: S	SEQ I	D NO	: 8:0						
CCAC	C AT	rg To et Cy 1	GC CT	rg Ar eu Me	NG CT	rg Ti eu Le 5	G CT	rg Cl	PA CT	eu As	AC CT	rg Gl	AG G(Lu A)	CT AC	CA nr	47
GTG Val 15	AAG Lys	GCA Ala	GCG Ala	GTA Val	CTC Leu 20	ATC Ile	CCT Pro	CAA Gln	AGT Ser	TCA Ser 25	GTG Val	TGT Cys	CCA Pro	AAC Asn	GCC Ala 30	95
GAG Glu	GCC Ala	AAT Asn	AAC Asn	TTT Phe 35	CTC Leu	CAG Gln	AAC Asn	GTG Val	AAG Lys 40	GTC Val	AAC Asn	CTG Leu	AAA Lys	GTC Val 45	ATC Ile	143

PCT/US96/11889 WO 97/04097

AAC Asn	TCC Ser	CTT Leu	AGC Ser 50	TCA Ser	AAA Lys	GCG Ala	AGC Ser	TCG Ser 55	AGA Arg	AGG Arg	CCC Pro	TCA Ser	GAT Asp 60	TAC Tyr	CTC Leu	191
						TGG Trp										239
AGA Arg	TAT Tyr 80	CCT Pro	TCT Ser	GTG Val	ATC Ile	TGG Trp 85	GAG Glu	GCA Ala	CAG Gln	TGC Cys	CGC Arg 90	CAC His	CAG Gln	CGC Arg	TGT Cys	287
						TTG Leu										335
						CTG Leu										383
ACT Thr	TTC Phe	CGG Arg	GTG Val 130	GAG Glu	AAG Lys	ATG Met	CTG Leu	GTG Val 135	GGC Gly	GTG Val	GGC Gly	TGC Cys	ACC Thr 140	TGC Cys	GTT Val	431
						GCG Ala		TAAT	raa							461

(2) INFORMATION FOR SEQ ID NO:4:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 150 amino acids (B) TYPE: amino acid

 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: protein
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Met Cys Leu Met Leu Leu Leu Leu Leu Asn Leu Glu Ala Thr Val Lys

Ala Ala Val Leu Ile Pro Gln Ser Ser Val Cys Pro Asn Ala Glu Ala 20 25 30

Asn Asn Phe Leu Gln Asn Val Lys Val Asn Leu Lys Val Ile Asn Ser 35 40

Leu Ser Ser Lys Ala Ser Ser Arg Arg Pro Ser Asp Tyr Leu Asn Arg 50 60

Ser Thr Ser Pro Trp Thr Leu Ser Arg Asn Glu Asp Pro Asp Arg Tyr

Pro Ser Val Ile Trp Glu Ala Gln Cys Arg His Gln Arg Cys Val Asm 85 90 95

Ala Glu Gly Lys Leu Asp His His Met Asn Ser Val Leu Ile Gln Gln

Glu Ile Leu Val Leu Lys Arg Glu Pro Glu Lys Cys Pro Phe Thr Phe

Arg Val Glu Lys Met Leu Val Gly Val Gly Cys Thr Cys Val Ser Ser

PCT/US96/11889 WO 97/04097

Ile Val Arg His Ala Ser

(2) INFORMATION FOR SEQ ID NO:5:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 459 base pairs

 - (B) TYPE: nucleic acid (C) STRANDEDNESS: double
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA
- (iii) HYPOTHETICAL: NO
- (ix) FEATURE:
 - (A) NAME/KEY: CDS
 - (B) LOCATION: 1:.453

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

				_			CTG Leu		48
							CCA Pro 30		96
							ACT Thr		144
							TAC Tyr		192
							CAA Gln	 	240
 	 	 	 -			 	GGA Gly	 	288
	 						CCT Pro 110	 	336

CAA GAG ATT CTA GTG GTG CGC AAA GGG CAT CAA CCC TGC CCT AAT TCA Gln Glu Ile Leu Val Val Arg Lys Gly His Gln Pro Cys Pro Asn Ser 115 120 125

TTT AGG CTA GAG AAG ATG CTA GTG ACT GTA GGC TGC ACA TGC GTT ACT

Phe Arg Leu Glu Lys Met Leu Val Thr Val Gly Cys Thr Cys Val Thr

384

432

- CCC ATT GTT CAC AAT GTA GAC TAAAAG Pro Ile Val His Asn Val Asp
- (2) INFORMATION FOR SEQ ID NO:6:
 - (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 151 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: protein
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Met Thr Phe Arg Met Thr Ser Leu Val Leu Leu Leu Leu Ser Ile 1 5 10 15

Asp Cys Ile Val Lys Ser Glu Ile Thr Ser Ala Gln Thr Pro Arg Cys 20 25 30

Leu Ala Ala Asn Asn Ser Phe Pro Arg Ser Val Met Val Thr Leu Ser 35 40 45

Ile Arg Asn Trp Asn Thr Ser Ser Lys Arg Ala Ser Asp Tyr Tyr Asn 50 55

Arg Ser Thr Ser Pro Trp Thr Leu His Arg Asn Glu Asp Gln Asp Arg 65 70 75 80

Tyr Pro Ser Val Ile Trp Glu Ala Lys Cys Arg Tyr Leu Gly Cys Val 85 90 95

Asn Ala Asp Gly Asn Val Asp Tyr His Met Asn Ser Val Pro Ile Gln
100 105 110

Gln Glu Ile Leu Val Val Arg Lys Gly His Gln Pro Cys Pro Asn Ser 115 120 125

Phe Arg Leu Glu Lys Met Leu Val Thr Val Gly Cys Thr Cys Val Thr 130 140

Pro Ile Val His Asn Val Asp 145 150

- (2) INFORMATION FOR SEQ ID NO:7:
 - (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 28 base pairs
 - (B) TYPE: nucleic acid(C) STRANDEDNESS: double
 - (D) TOPOLOGY: linear
 - (ii) MOLECULE TYPE: cDNA
 - (iii) HYPOTHETICAL: NO
 - (xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

CACAGGCATA CACAGGAAGA TACATTCA

- (2) INFORMATION FOR SEQ ID NO:8:
 - (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 25 base pairs (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: double
 - (D) TOPOLOGY: linear
 - (ii) MOLECULE TYPE: cDNA

WO 97/04097			PCT/US96/11889
(iii) HYPOTHETICAL:	: NO		
(xi) SEQUENCE DESC	CRIPTION: SEQ ID NO:	8:	
TCTTGCTGGA TGGGAACGGA	ATTCA		25
(2) INFORMATION FOR SE	EQ ID NO:9:		
(B) TYPE: nu	28 base pairs ucleic acid DNESS: double		
(ii) MOLECULE TYPE	E: cDNA		
(iii) HYPOTHETICAL:	: NO	ē.	, •
(xi) SEQUENCE DESC	CRIPTION: SEQ ID NO:	9:	
ATACATTCAC AGAAGAGCTT	CCTGCACA		28

5 What is claimed is:

- 1. An isolated polynucleotide comprising a nucleotide sequence selected from the group consisting of:
- (a) the nucleotide sequence of SEQ ID NO:1 from nucleotide 146 to nucleotide 544:
- (b) a nucleotide sequence capable of hybridizing to a nucleic acid sequence specified in (a);
 - (c) a nucleotide sequence varying from the sequence of the nucleotide sequence specified in (a) as a result of degeneracy of the genetic code; and
 - (d) an allelic variant of the nucleotide sequence specified in (a).

15

20

30

- 2. The polynucleotide of claim 1 wherein said nucleotide sequence encodes a protein having CTLA-8 activity.
- 3. The polynucleotide of claim 1 wherein said nucleotide sequence is operably linked to an expression control sequence.
 - 4. The polynucleotide of claim 1 comprising the nucleotide sequence of SEQ ID NO:1 from nucleotide 55 to nucleotide 544.
- 5. A host cell transformed with the polynucleotide of claim 3.
 - 6. The host cell of claim 5, wherein said cell is a mammalian cell.
 - 7. A process for producing a human CTLA-8 protein, said process comprising:
 - (a) growing a culture of the host cell of claim 5 in a suitable culture medium; and
 - (b) purifying the human CTLA-8 protein from the culture.

5 8. An isolated human CTLA-8 protein comprising an amino acid sequence selected from the group consisting of:

(a) the amino acid sequence of SEQ ID NO:2;

10

20

25

- (b) the amino acid sequence of SEQ ID NO:2 from amino acids 11 to 163;
- (c) the amino acid sequence of SEQ ID NO:2 from amino acids 29 to 163;
- (d) the amino acid sequence of SEQ ID NO:2 from amino acids 31 to 163; and
- (e) fragments of (a), (b), (c) or (d) having CTLA-8 activity.
- 9. The protein of claim 8 comprising the amino acid sequence of SEQ ID NO:2.
- 15 10. The protein of claim 8 comprising the sequence from amino acid 29 to 163 of SEQID NO:2.
 - 11. A pharmaceutical composition comprising a human CTLA-8 protein of claim 8 and a pharmaceutically acceptable carrier.

12. A human CTLA-8 protein produced according to the process of claim 7.

- 13. A composition comprising an antibody which specifically reacts with a human CTLA-8 protein of claim 8.
- 14. A method of treating a mammalian subject comprising administering a therapeutically effective amount of a composition of claim 11.
- 15. A method of treating a mammalian subject comprising administering a therapeutically effective amount of a composition comprising a pharmaceutically acceptable carrier and a protein comprising an amino acid sequence selected from the group consisting of:
 - (a) the amino acid sequence of SEQ ID NO:4;

(b) the amino acid sequence of SEQ ID NO:4 from amino acids 18 to 150; and

- (c) fragments of (a) or (b) having CTLA-8 activity.
- 16. The method of claim 15 wherein said protein comprises the amino acid sequence of SEQ ID NO:4.

17. The method of claim 15 wherein said protein comprises the amino acid sequence of SEQ ID NO:4 from amino acids 18 to 150.

- 18. A method of treating a mammalian subject administering a therapeutically effective amount of a composition comprising a pharmaceutically acceptable carrier and a protein comprising an amino acid sequence selected from the group consisting of:
 - (a) the amino acid sequence of SEQ ID NO:6;
 - (b) the amino acid sequence of SEQ ID NO:6 from amino acids 19 to 151; and
 - (c) fragments of (a) or (b) having CTLA-8 activity.

20

25

5

- 19. The method of claim 18 wherein said protein comprises the amino acid sequence of SEQ ID NO:6.
- 20. The method of claim 18 wherein said protein comprises the amino acid sequence of SEQ ID NO:6 from amino acids 19 to 151.
 - 21. The polynucleotide of claim 1 comprising the nucleotide sequence of SEQ ID NO:1 from nucleotide 86 to nucleotide 544.
- The protein of claim 8 comprising the sequence from amino acid 11 to 163 of SEQ ID NO:2.

5 23. A method of treating a mammalian subject comprising administering a therapeutically effective amount of a composition comprising a pharmaceutically acceptable carrier and IL-17 or an active fragment thereo.

- 24. The method of claim 14, 15, 18 or 23 wherein said subject is treated to produce an effect selected from the group consisting of inhibition of angiogenesis, inhibition of growth or proliferation of vascular endothelial cells, inhibition of tumor growth, inhibition of angiogenesis-dependent tissue growth, proliferation of myeloid cells or progenitors, proliferation of erythroid cells or progenitors, proliferation of lymphoid cells or progenitors, induction of IFNγ production, induction of IL-3 production and induction of GM-CSF production.
 - 25. The composition of claim 3 wherein said polynucleotide is contained in a vector suitable for *in vivo* expression in a mammalian subject.
- 26. The polynucleotide of claim 1 comprising the nucleotide sequence of SEQ ID NO:1 from nucleotide 139 to nucleotide 544.
 - 27. The protein of claim 8 comprising the sequence from amino acid 31 to 163 of SEQ ID NO:2.

133 \$1 51 53 0.0.0 VVRKGHOPCPNSF VLKRBPEKCPFTF VVRRKHOGGSVSF လ လ လ E- E- E-SKRASDYNNRS SRRPSDYLNRS SR---NIESRS 7 1 8 1 7 1 8 1 7 1 8 ٠, လ လ ≭ FKS SSE NXX VXX 000 000 1111 200 200 , ... z ဖြော STIRING IN STIRING တတလ GIN VD YHHNS SGKLDHHHNS JGKEDISHNS FILONNY WILK --SHKKLDIG N A D N N A B O RYLGCVN RHORCVN S Z ~ W D. V T P I V H N V D V S S I V R H A S V T P V I H H V Q 4 4 > SSVIWEAKICR SSVIWEAQCR SEVVQAQCR 1000 1000 1000 န္ ဟ မ **€** 00 ₽ F P A 400 000 ខាម >->-E- E- E-A A R KITPKN CHTF **∞** ∞ ∞ 000 O O O 000 RLEKHLVTVG RVEKHLVGVG QLEKVLVTVG WTLSRNED WTLSRNED WNYTVTW Hsvie_2 Husctla8 B18_F1 Hsvie_2 Husctla8 B18_F1 Hsvie_2 Musccla8 B18_F1

FIGURE

2/7

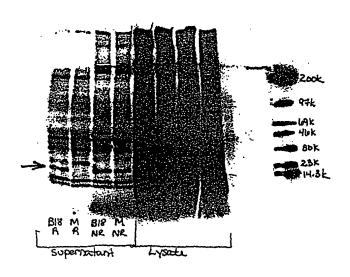


FIGURE 2

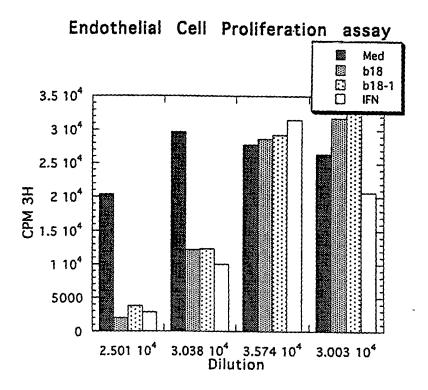


Fig. 3

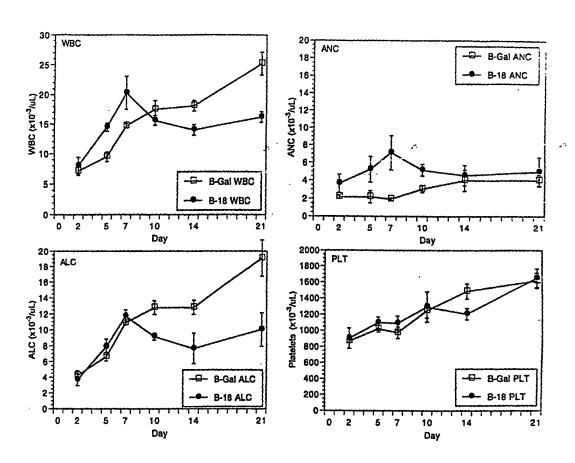


Fig. 4

TF-1 activity in CM from B18 induced HS-5 cells

